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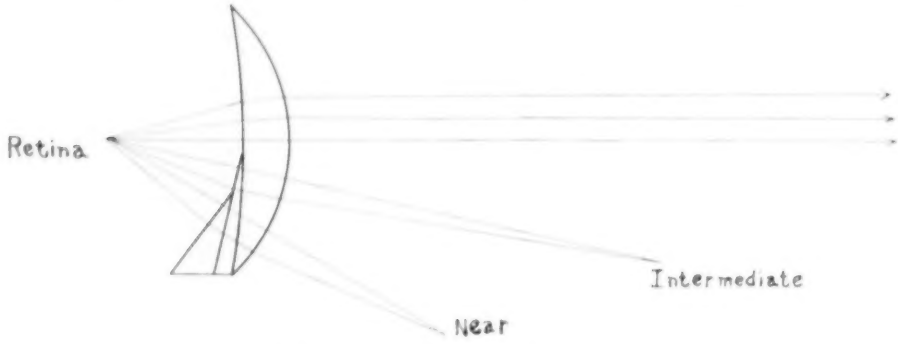

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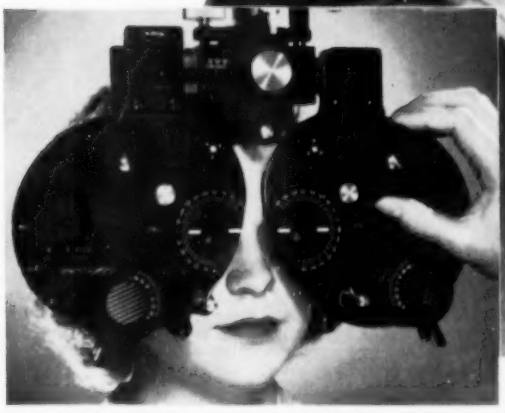

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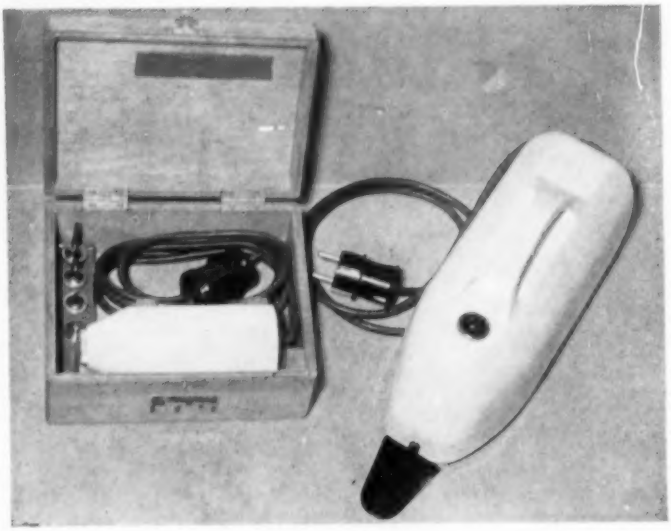

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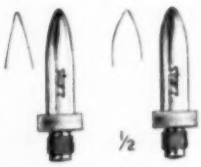

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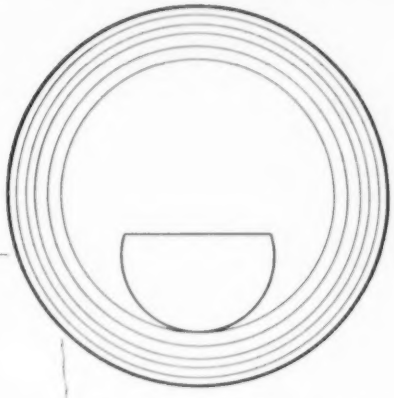

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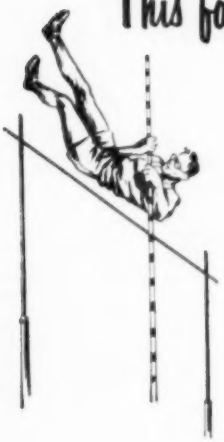

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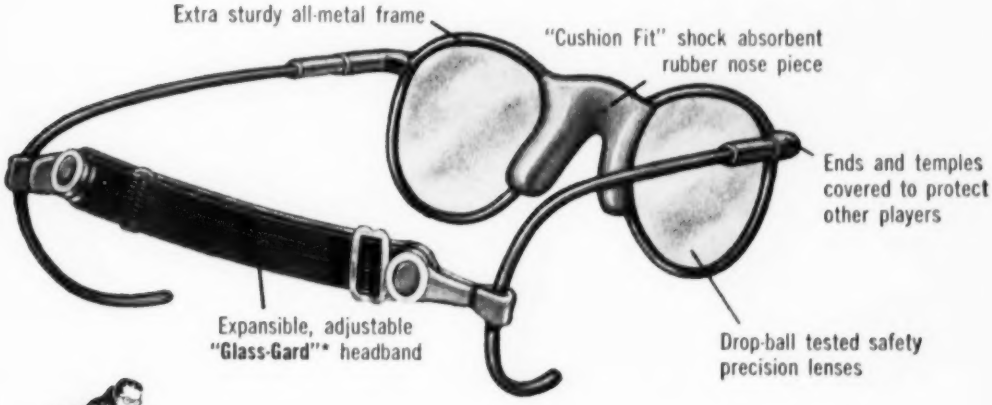
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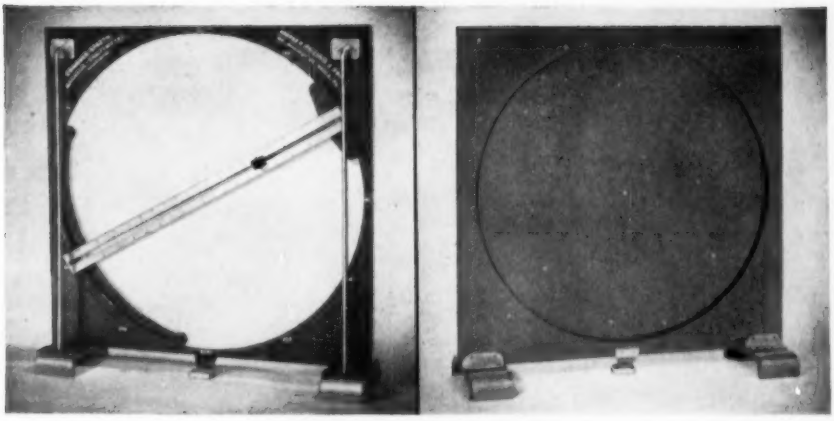

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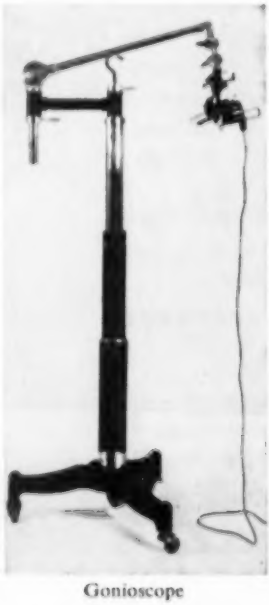

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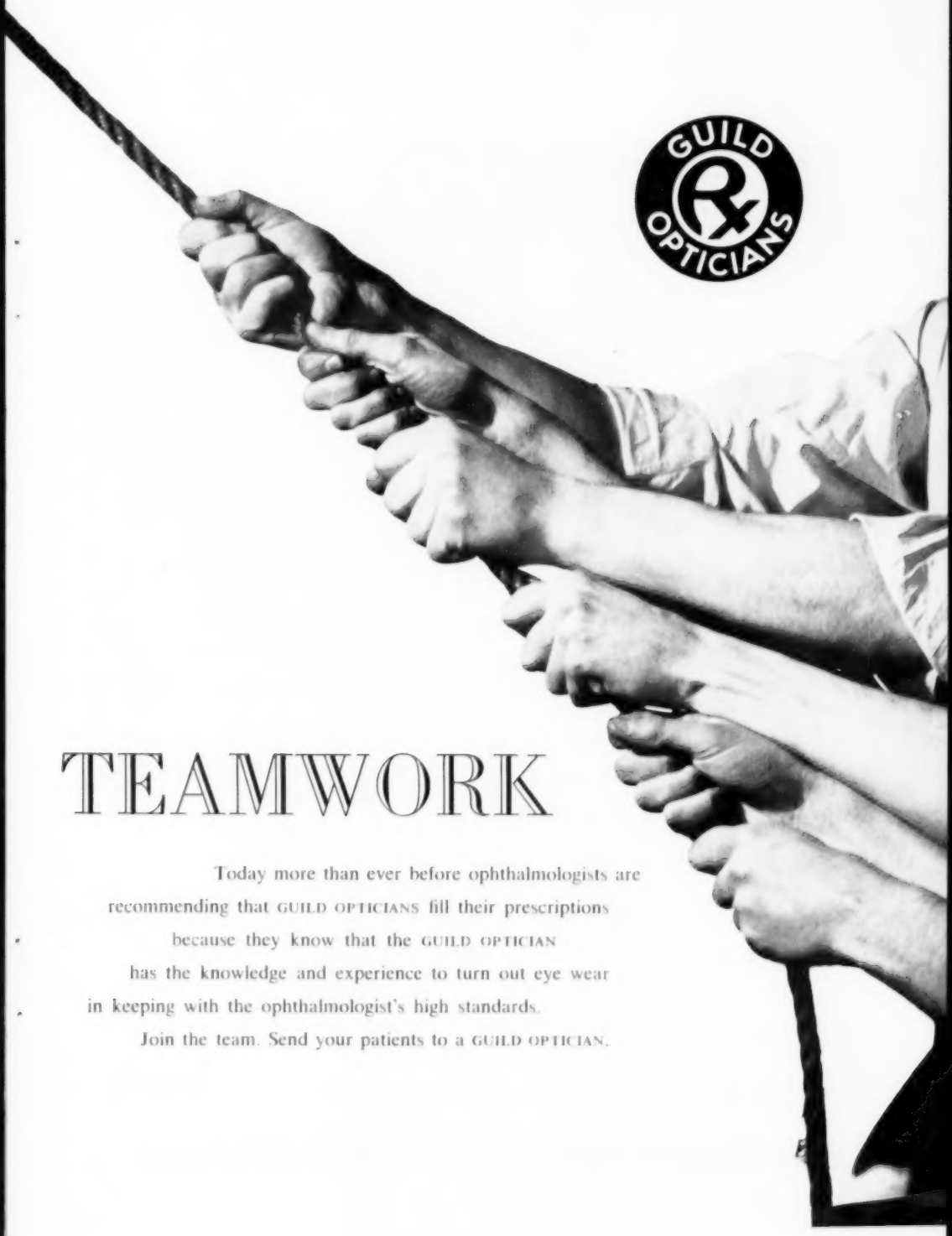

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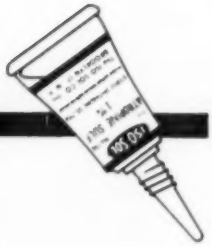

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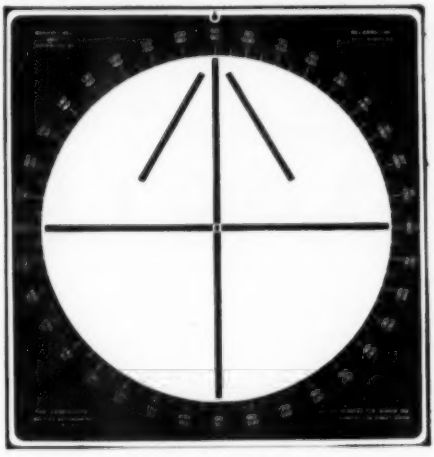

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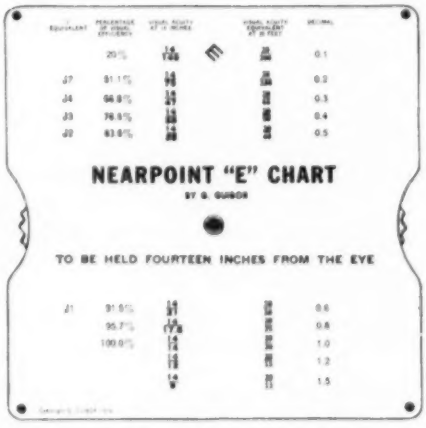

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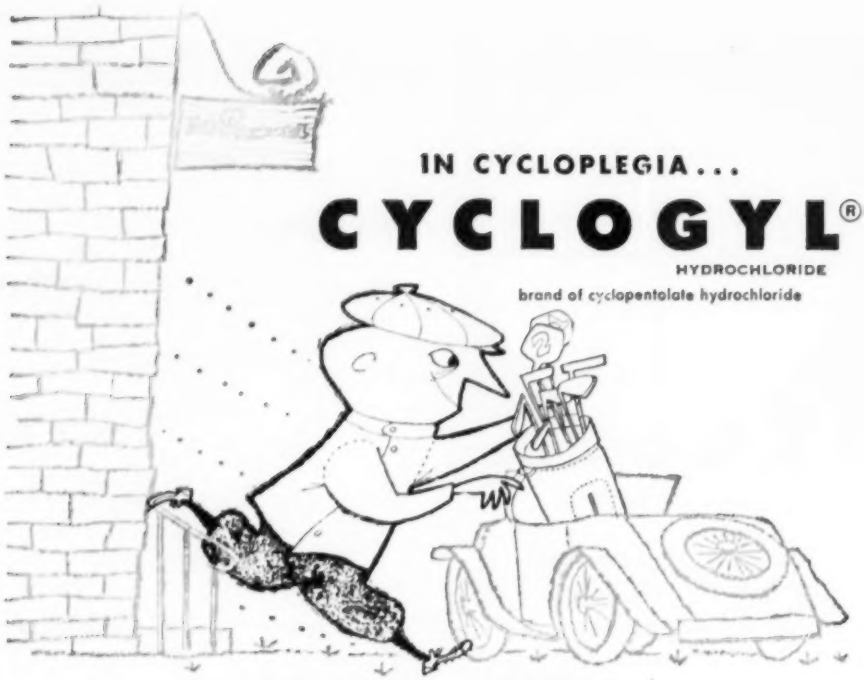

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1. Gordon, D. M., and Ehrenberg, M. H.: Am. J. Ophth. 38:831 (Dec.) 1954.

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Diagnosis of Malignant Melanoma by Subretinal Fluid Studies

LEONARD CHRISTENSEN, M.D.
and
GERALD E. ROWEN, M.D., Portland, Ore.

Most malignant melanomas of the choroid manifest themselves as easily recognized, solid, pigmented subretinal masses. Occasionally these tumors are masked by detachment of the retina and are discovered only after long delay and potentially dangerous therapy. Consequently, several techniques have been devised to differentiate malignant melanoma from other elevated lesions of the fundus, but none has been wholly satisfactory.* Diagnosis by microscopic study of aspirated subretinal fluid is one of these methods. This is an application of Papanicolaou's technique for the detection of carcinomas in other tissues, in which diagnosis is made by the appearance of individual tumor cells or clumps of cells in tissue fluids.[5] This method has applicability because in most malignant tumors there are a sparse stromal network and poor cellular cohesion, which permit desquamation of the surface cells. Theoretically, intraocular malignant melanomas should be ideal for such a diagnostic procedure, since the surface of this tumor is bathed by subretinal fluid trapped within an enclosed space. Nevertheless, the technical difficulties and possible dangers have kept this technique from receiving wide acceptance.†

The purpose of the present study was to determine the frequency with which free-floating tumor cells appear in subretinal fluid, the ease with which they can be recognized, and the possible dangers of the procedure. The study consisted of the following steps: (1) microscopic examination of globes containing a malignant melanoma and of subretinal fluid obtained from globes having either idiopathic retinal detachment or detachment secondary to tumor; (2) examination of prints made from the cut surface of ocular malignant melanomas, as well as other malignant tumors, to study individual

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From the Department of Ophthalmology, University of Oregon Medical School.

Presented at the 39th Annual Meeting of the Pacific Coast Oto-Ophthalmological Society, May 19, 1955, at Portland, Ore.

* References 1 to 4.

† References 6 to 9.

Fig. 1.—Retina overlying inner surface of tumor, and Bruch's membrane and pigment epithelium over pedicle, which isolate this tumor from subretinal fluid.

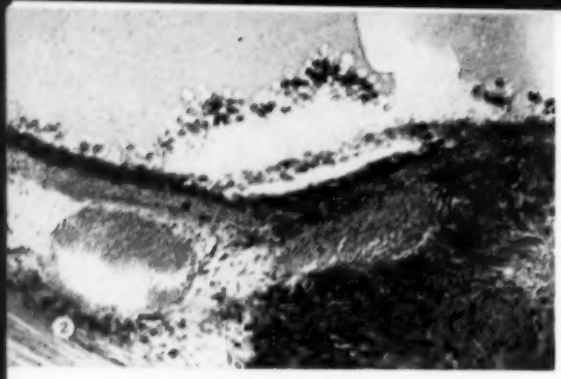
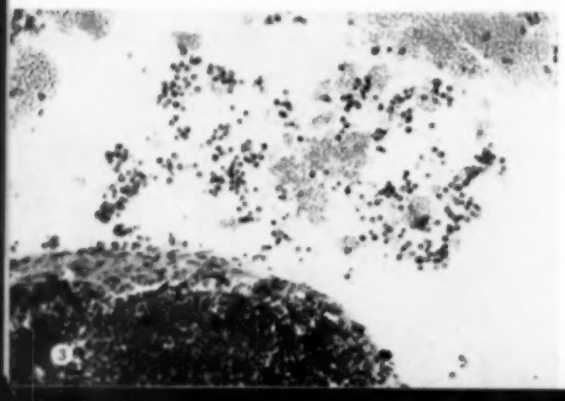


Fig. 2.—Pigment epithelial cells desquamating into subretinal fluid. This eye contained a malignant melanoma.

tumor cells and compare the cohesiveness of the cells, and (3) microscopic examination of globes for any visible effect on growth of the tumor from diathermy applied prior to enucleation.

Microscopic sections of globes containing an intraocular malignant melanoma most frequently show a tumor bathed by fluid beneath a detached retina. The tumor, however, is of choroidal origin, and the surface that extends into the vitreous cavity is usually covered by Bruch's membrane, pigment epithelium, or a membrane formed by metaplasia of the pigment epithelium. The surface is exposed only in the area in which Bruch's membrane is ruptured, and then it may be covered with overlying retina; consequently, there is often little direct contact of the fluid with tumor cells (Fig. 1). Nevertheless, of 83 specimens examined, we were able to demonstrate tumor cells in the subretinal coagulum in 45. The number of melanoma cells varied according to type of tumor. Spindle-cell Types A and B showed less tendency to shed cells, in contrast to the epithelioid type. Of 5 tumors classified as necrotic, all showed free-floating tumor cells, as well as 13 out of 16 classified as epithelioid. Since these microscopic sections were only 20μ thick and the cells were not concentrated by centrifuging, it is reasonable to assume that the actual incidence of free-floating cells within the fluid was much greater than the number found.

Fig. 3.—Low-power magnification of tumor cells.



Subretinal fluid was obtained on four occasions prior to the establishment of a diagnosis. In three of these specimens we were able to identify melanoma cells. The greatest difficulty was in differentiating tumor cells from histiocytes. Desquamated cells released from pressure of the tumor assume a more rounded contour and appear similar to free-floating pigment epithelial cells, which are also present. When pigment epithelium is disturbed, as in retinal detachments and inflammations, individual cells separate from the parent epithelium and migrate into subretinal space (Fig. 2). They may retain or discard their pigment and assume the role of macrophages. They appear as large round cells with single nuclei, whose cell detail is often obscured by pigment. Because of this pigmentation and similarity in appearance, differentiation of tumor cells from macrophages was difficult, and often impossible (Figs. 3 and 4). Where tumor cells were relatively free of pigment and appeared in clumps, recognition was simple, but existence in this form was not common.

A characteristic common to all malignant tumors is reduced cohesiveness of the parenchymal cells of the tumor. It is this feature that makes possible successful application of the Papanicolaou technique. This has previously been noted and reported for malignant melanomas occurring in the skin and other organs.‡ However, no specific data are available in the American literature concerning intraocular malignant melanomas. It was desirable, therefore, to obtain prints of the cut surface of malignant melanomas and other carcinomas for purposes of studying cell structure and comparing the relative cohesiveness of these cells in various tumors. Since most of the material received in the Weeks Memorial Laboratory at the Uni-

‡ References 10 and 11.

versity of Oregon Medical School is already fixed in formalin or other fixatives, most of the prints were made on fixed tissue. This procedure consisted of pressing a clean slide against the cut surface of the tumor and then staining with hematoxylin and eosin. This procedure was followed for intraocular malignant melanomas, retinoblastomas, and carcinomas of the breast, cervix, uterus, and lymph nodes. Of the fixed tissues studied, intraocular malignant melanomas shed the fewest cells. However, a print made from the cut surface of a fresh melanoma was richly studded with tumor cells. Consequently, it was apparent that any comparative study utilizing fixed tissue was not valid because of error induced by fixation. On the other hand, the cytologic appearances of the individual cells in fixed versus unfixed tissue showed no appreciable difference. The most striking finding in the cell study was a wide variation in the morphology among cells of different melanomas. Cell shapes ranged from ovoid to round, and there were marked differences in the nuclear-cytoplasmic ratio and cell size. The chromatin arrangement also varied from fine nuclear stippling to solid intranuclear masses.

The above study demonstrated that freely floating tumor cells often were present in the subretinal fluid, but positive recognition was difficult. It would seem that microscopic study of the subretinal fluid would be of diagnostic value if it were safe. Four specimens with malignant melanoma were available for study in which diathermy had been used. In three globes containing malignant melanomas, penetrating diathermy had been applied either as treatment for retinal detachment or for removal of subretinal fluid. In these globes, extraocular extension of the tumor had taken place. In the first, there was a period of two and one-half years between retinal detachment surgery and enucleation. The last 18 months of this period was characterized by recurrent bouts of pain, increased tension, and complete loss of vision. At enucleation, tumor tissue was

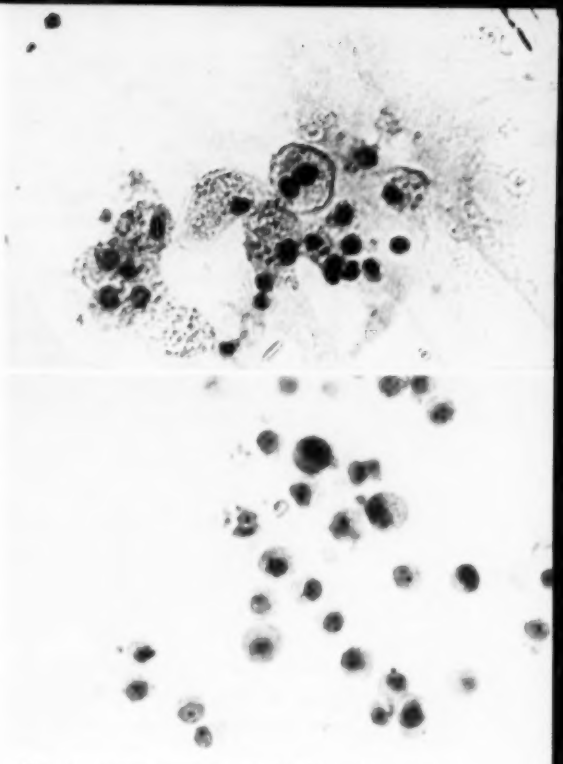


Fig. 4.—Above, tumor cells, × 825; below, similarity of tumor cells, above, to histiocytes, below.

Fig. 5 (Case 2).—Low-power magnification, showing extension of tumor through sclera.

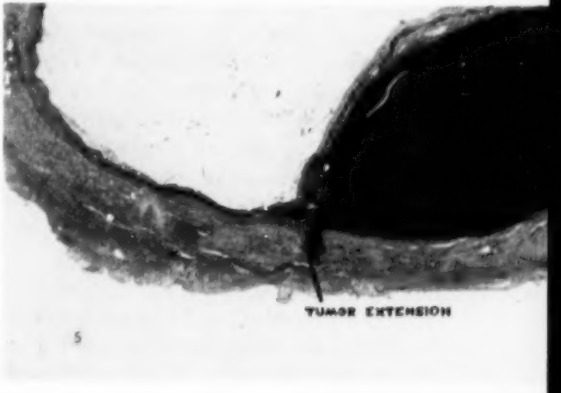


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Fig. 6 (Case 3).—Low-power view, showing extension of tumor through sclera.

6

found lying free within the orbital cavity. In the second, there were multiple diathermy punctures in the sclera overlying the tumor. The period between detachment surgery and enucleation in this case was six weeks. Extraocular extension occurred, but only through the puncture that had penetrated the choroid (Fig. 5). In the last case, a retinal detachment was suspected of masking an underlying malignant melanoma. A diagnostic diathermy puncture was placed in the region of the detachment and subretinal fluid aspirated. Smears of this fluid showed the presence of pigmented cells, but differentiation between phagocytes containing pigment and tumor cells was impossible and the smear was reported negative. The true nature of the disturbance became apparent on subsequent clinical examination, and the eye was enucleated 13 days following application of diathermy. Microscopic sections of this specimen show extraocular extension of the tumor through the diathermy puncture (Fig. 6). Obviously, externalization of the tumor had taken place in this 13-day period. In a fourth specimen, surface diathermy had been applied a year prior to enucleation, and microscopic sections of this specimen showed a localized area of destruction of tumor within the globe but no extraocular extension. Integrity of the sclera had not been disrupted by the original surgical procedure. Unfortunately, no specimens were available for study in which fluid was obtained through needle puncture.

SUMMARY AND CONCLUSIONS

In a microscopic study of subretinal fluid in globes with retinal detachment secondary to malignant melanoma, a high incidence of free-floating tumor cells was found. These cells may be recovered by aspiration of fluid and can be positively identified by microscopic examination provided a microscopist exceptionally well versed in the cellular characteristics of subretinal fluid is available. Failure to identify the presence of a tumor by this method has promoted subsequent externalization of a previously confined neoplasm. Because of the technical difficulties involved and the obvious danger of extraocular extension of unrecognized tumors, this procedure is not recommended as a diagnostic aid.

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The Treatment of Conical Cornea

Introduction of A New Multicurve Fluidless Corneal Contact Lens

THEODORE N. ZEKMAN, M.D.
and
BURTON M. KRIMMER, M.D., Chicago
With the Technical Assistance of Newton K. Wesley, O.D., and Evelyn Corral

The subject of keratoconus is submerged in the literature of the development of contact lenses, which has appeared for the most part in foreign publications. The early history of contact lenses per se has been carefully reviewed in English by Obrig [1] and others * up to and including 1938. In the interests of ready reference to keratoconus, some developments may be highlighted herewith.

The first effort toward the optical improvement of keratoconus dates back to 1889, when Kalt † designed a blown-glass, fluidless corneal lens without a scleral flange. Its success was limited, however, because of the poor optical qualities of the glass, difficulties in fitting, and the excessive weight of the lens. Zeiss ‡ and Müller § brought about a return to the more conventional fluid lens with the supporting scleral flange. There was a continuing effort to improve the lenses, both optically and physiologically. It was not until 1932, when Dallos [6] introduced the use of Negacoll for making direct molds of the eye, that the construction of an accurately fitting contact lens became a possibility. The Dallos glass lenses had many disadvantages, chief of which were difficulty in manufacture, excessive weight, breakage, and poor optical qualities. In 1936 Feinbloom introduced plastic contact lenses in an effort to reduce their weight.[7] At first the scleral rim was made of plastic, but in the same year Obrig [8] introduced the first all-plastic, molded contact lens, constructed of a synthetic resin, polymethylmethacrylate. This lens was widely employed because of the advantages of accuracy of optical qualities, lightness, and resistance to breakage.

Problems resulting from the abnormal physiologic state produced in the eye by wearing fluid lenses still had to be solved. Patients fitted with fluid lenses found that they could wear them for only a limited time because of the absorption of the artificial lens fluid into the cornea, which produced epithelial edema and clouding of vision. Other disadvantages, as described in a comparative study of fluid and fluidless lenses by the Army Medical Research Laboratory group,[9] were photophobia, chromatic halo around lights, impairment in visual acuity, and development of entoptic field changes.

It was finally recognized, after extensive research, that the normal metabolism of the cornea required an adequate oxygen supply and means of excretion of carbon dioxide.[10] This necessitated a free circulation of blood at the limbus and the free flow of tears over the corneal surface. Nugent's first report [11] of the Tuohy corneal lens appeared in 1948. This lens has no scleral flange and utilizes the patient's tears instead of an artificial fluid as the fluid layer between lens and cornea. The success of this lens has been verified in studies of Berens [12] and McGraw.[9] Since the advent of the Tuohy lens, several other fluidless contact lenses have been developed: the Dallos ventilated glass lens, the Lacrilens of Obrig, the scleral fluidless lens of Mueller-Welt, the Para-Scleral lenses produced by

Submitted for publication June 24, 1955.

From the Departments of Ophthalmology, Michael Reese Hospital and Cook County Hospital.

* References 2, 3, and 4.

† Obrig,[1] p. 129.

‡ Cited in Sulzer.[5]

§ Cited by Sitchevska.[3]

Butterfield, the Feinbloom self-centering lens, and the PCL (plastic contact lens), produced by Wesley and Jessen.[2] Not all these lenses have been reported in the literature, and little is known regarding their practical application other than what is published in the manufacturers' brochures.

In 1952, Berens [12] reported the cases of 54 patients fitted with the Tuohy lens. Only four of these patients had keratoconus, and all had excellent results. In McGraw and Enoch's [8] comparative study of the fluid lens, the Tuohy lens, the Dallos lens, and the Lacrilens, there were no cases of keratoconus, and the Dallos lens and the Lacrilens proved superior in performance but were difficult to fit. The fluid lens was easiest to fit but had limited wearing time because of the development of corneal edema. The Tuohy lens was easily fitted but was associated with protracted adaptation time and was easily dislodged.

The few extensive studies made to date on the effect of contact lenses in a large series of patients with keratoconus state that persons with keratoconus respond favorably no matter what type of contact lens is worn—fluid or fluidless. We are in disagreement with this view and feel that the enthusiasm expressed by earlier observers resulted from the tremendous gratification derived by any keratoconus patient who is given visual acuity not experienced prior to wearing contact lenses. In most surveys on the effect of contact lenses, it is usually the patient with keratoconus who is thought to be the most benefited. It was this misconception that prompted the present study. We have observed harmful effects of certain types of contact lenses, especially the fluid lens. We are, therefore, introducing a newly designed, fluidless contact lens for patients with medium and severe keratoconus. In incipient cases of keratoconus in which the corneal base curve is 7.75 mm. or flatter, the eye can usually be fitted successfully with stock fluidless lenses of various types now available.

Inasmuch as most medium and severe cases of keratoconus have a corneal curvature of 7.0 mm. or less, it soon became obvious that the conventional type of single-curve fluidless corneal contact lens could not be worn successfully. These lenses became easily dislodged because of the poor adaptation of the single-curve lens to the conical cornea, with multiple curves. Invariably, there was gaping in the periphery of the lens. To obviate this difficulty, a multicurve fluidless corneal contact lens was developed by Wesley and Jessen.[2]

NEW DESIGNS

Tricurve Lens: Applicable Measurements

At first, a tricurve lens (Fig. 1) was developed with three inside curves, a central and two peripheral. A small 1.2 mm. bevel was added to the periphery. The central curve is the most acute, whereas the adjacent curve is 0.37 mm. flatter, and the peripheral, or third, curve is, again, 0.37 mm. flatter than the second. The radius of the central curve is 7.0 mm., whereas the adjacent curves have radii of 7.37 and 7.75 mm., respectively. It was found that this lens fitted many patients with incipient and most patients with medium keratoconus.

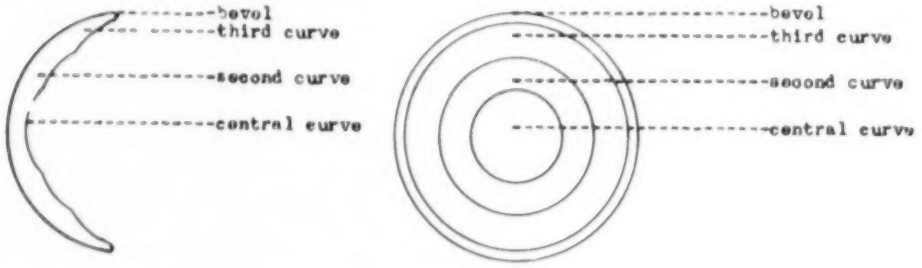


Fig. 1.—Tricurve lens.

Multicurve Lens for Extreme Keratoconus: Proper Measurements

The Tricurve lens was not successful in patients with extreme keratoconus, because the central corneal curvature was too acute, being in the range of 6.50 mm., and there were more than three peripheral corneal curves. In 1951 a Universal Multicurve lens (Fig. 2) was therefore devised. This lens has a central curvature of 6.50 mm. with five peripheral curves, measuring 7.00, 7.50, 8.00, 8.50, and 9.00 mm. The 1.2 mm. bevel at the edge, which aids in support of the lens, was retained.

Fitting

The measurements given have been used in the Tricurve and Multicurve lenses because experience proved them to be the most applicable. Both the Tricurve and the Multi-

Fig. 2.—Universal Multicurve lens.

curve lens can be ground to any desirable curve. The fitting of the lenses is similar to that of other types of fluidless lenses. It is recommended that these lenses be fitted by a technician skilled in proper technique, working under the supervision of an ophthalmologist.

Advantages of New Designs

The advantages of the two lenses described in the treatment of keratoconus lie in their extreme lightness and close conformity to the irregular corneal surface. They are made of optical quality polymethylmethacrylate. They exert some restraint on the cone and, indeed, actually produce a diminution in the size of the cone.

Study of Twenty-Nine Patients with Keratoconus Wearing New Multicurve Lenses

Method of Study

We have conducted an analytical investigation involving 29 patients who are wearing Multicurve corneal lenses because of a severe degree of keratoconus. Eighteen of these patients formerly wore fluid lenses prior to receiving the corneal lenses from 1 to as long as 14 years. The average length of time that this group had worn fluid lenses was 7.1 years. The remaining 11 patients have worn only the corneal lens. Two patients in the entire group have worn corneal lenses for as long as 3.5 years; however, the average wearing time of corneal lenses at this writing (May, 1955) is 2.1 years. Although the group wearing corneal lenses has not been followed for so long a period as the group with fluid lenses, the changes as a result of wearing the new fluidless lens appear to be significant enough to warrant a report at this time. The study, nevertheless, is being continued, and new patients are being added as they occur.

The patients included in the survey have been examined by several ophthalmologists, who permitted the records of these patients to be studied and used in this report. All patients were fitted for fluidless contact lenses by the same technician, Evelyn Corral.

Significant Factors in Evaluation

Several factors were significant in evaluating the Multicurve lens: (1) changes in visual acuity in the group with fluidless lenses who had previously worn fluid lenses; (2) improvement in visual acuity in patients with fluidless lenses without prior treatment with fluid lenses; (3) incidence of corneal hydrops; (4) corneal vascularization in keratoconus; (5) comparative subjective symptoms of patients with fluid and fluidless lenses, and (6) comparative wearing ability of fluid versus fluidless lenses.

1. *Changes in Visual Acuity in Group with Fluidless Lenses; Fluid Lenses Previously Worn.*—As will be seen in Tables 1 and 2, improvement in visual acuity occurred in slightly more than 68% of the patients. If one considers this in terms of individual eyes, it

TABLE 1.—*Change in Visual Acuity After Wearing Fluidless Lenses in Patients with Previous Fluid Lenses*

Case	Fluid Lenses, Years Worn	Before		After		Fluidless Lenses, Years Worn
		O. D.	O. S.	O. D.	O. S.	
1	14	0.2	0.2	0.5	0.5	2.5
2	13	0.8	0.2	0.5	0.15	3.0
3	12	0.2	0.3	0.5	0.5	1.5
4	11	0.7	0.7	0.8	0.8	2
5	11	0.5	0.5	0.5	0.5	2
6	11	0.3	0.2	0.5	0.5	2
7	11	0.4	0.4	0.85	0.85	2
8	10	0.3	0.25	0.3	0.25	2.5
9	8	0.7	0.7	0.9	0.85	2.25
10	7	0.8	1.0	0.8	0.85	3.0
11	7	0.3	0.5	0.5	0.7	2.5
12	7	0.4	0.5	0.4	0.5	2.0
13	5	0.3	0.4	0.3	0.2	2.0
14	3.5	0.3	0.2	0.5	0.5	2.0
15	3	0.1	0.15	0.9	0.9	2.5
16	2	1.0	1.0	1.0	1.0	3.5
17	2	0.1	0.2	0.9	0.6	2
18	1.5	0.3	0.2	0.6	0.5	2.5

will be noted that 22 eyes showed an increase in vision, 10 showed no change, and only 4 eyes decreased in visual acuity. These figures take on added importance when one observes, as shown in Table 3, that in this group of 18 patients, or 36 eyes, there were 24 cases of corneal hydrops. More will presently be said concerning hydrops of the cornea. It is emphasized now that, notwithstanding the injurious effect of hydrops on the transparency of the cornea, visual improvement was observed in 22 eyes and no further decrease was seen in 10 eyes. This suggests that the corneal lens prevented further scarring of the cornea and in most cases produced subsidence of corneal haze. It is our clinical impression that this is accomplished by pressure of the lens on the cornea, which actually reduces the amount of corneal edema.

2. *Improvement in Visual Acuity in Patients with Fluidless Lenses; No Prior Treatment with Fluid Lenses.*—Tables 4 and 5 reveal that 11 patients, or 22 eyes, all demonstrated an improvement in visual acuity. In most cases this improvement was far more striking than in the group of patients who had previously worn fluid lenses, probably owing to the absence of corneal hydrops among this group, so that the corneas remained transparent. During the follow-up study there has been no sign of visual loss in any of these patients.

3. *Incidence of Corneal Hydrops.*—As has been mentioned, 10 of the 18 patients who had worn fluid lenses had had attacks of corneal hydrops. In this group, all but two had more

TABLE 2.—*Per Cent of Change in Visual Acuity in Patients with Fluidless Lenses; Fluid Lenses Previously Worn*

	Loss of V. A.	No Change in V. A.	Improvement in V. A.	Total
No. of eyes	4	10	22	36
Change in visual acuity, %	11	21	68	100

TABLE 3.—*Incidence of Corneal Hydrops in Patients with Fluid Lenses*

No. of attacks	0	1	2	3	4
No. of patients	8	2	4	2	2
Total	18				

TABLE 4.—*Change in Visual Acuity in Patients with Only Fluidless Lenses*

Case	Fluidless Lenses, Years Worn	Before Lenses		After Lenses	
		O. D.	O. S.	O. D.	O. S.
1	1.0	0.5	0.5	0.7	Transplant
2	1.0	0.5	0.5	0.9	0.9
3	1.5	0.4	0.4	1.0	1.0
4	1.5	0.5	0.35	0.9	0.9
5	2.0	0.2	0.3	1.0	1.0
6	2.0	0.1	0.3	0.8	0.7
7	2.0	0.1	0.08	0.4	0.7
8	2.0	0.2	0.6	1.0	1.0
9	2.5	Not recorded		1.0	1.0
10	3.0	0.1	0.8	0.9	1.0
11	3.5	0.1	0.8	0.8	0.8

TABLE 5.—*Improvement in Visual Acuity in Patients with Only Fluidless Lenses*

	Loss of V. A.	No Change in V. A.	Improvement in V. A.	Total
No. of eyes	0	0	22	22
Per cent of eyes	0	0	100	100

TABLE 6.—*Incidence of Corneal Hydrops with Fluid Lenses*

Fluid lenses, years worn	1-5	6-10	11-15
Corneal hydrops, no. of attacks	4	8	12

than one attack; two had three attacks, and one patient had had four episodes of corneal hydrops. There was a total number of 24 episodes of corneal hydrops among the 10 patients. From Table 6 it is evident that those patients who wore fluid lenses longer had a higher incidence of hydrops.

Only 1 in the entire series of 29 patients fitted with corneal lenses has thus far developed hydrops of the cornea. This patient had previously worn fluid lenses for 11 years without any episodes of hydrops. Fluidless lenses were fitted because of subjective symptoms (photophobia, tearing, pain, blurring of vision) with the fluid lenses and because of the persistent progression of the keratoconus bilaterally with fluid lenses. The attack occurred in the right eye three months after the corneal lenses were fitted. Prior to the attack, the vision with corneal lenses increased from 0.5 to 0.7 in each eye. The attack lasted one month, during which pressure dressings were applied. The vision in the right eye dropped to 0.5. When the attack subsided, the patient resumed the wearing of corneal lenses and has been wearing them from 12 to 18 hours a day for the last year and a half without any untoward incident. A recent determination of visual acuity reveals that each eye, again, has 0.7 visual acuity.

4. *Corneal Vascularization in Keratoconus; Curvature Measurements.*—Vascularization: In patients with severe conical cornea who have had repeated episodes of acute hydrops of the cornea and who have worn fluid lenses for a prolonged period, the occurrence of vascularization of the cornea is not infrequent. Vascularization is both superficial and deep in the corneal stroma and regresses when the patient does not wear the fluid lens. Figure 3 well illustrates such an occurrence of vascularization in a patient with severe conical cornea who wore fluid lenses.

Fig. 3.—Vascularization in patient with severe conical cornea. Patient had worn fluid lenses.

Fig. 4.—Corneal molds used for measuring corneal radius.

All patients with corneal vascularization had severe bilateral keratoconus, and all but one had had previous episodes of corneal hydrops (Table 7). The corneal molds (Fig. 4) were repeated for these patients after they had worn fluidless lenses for a minimum of two years. In three patients the lenses were worn for somewhat longer periods.

Curvature Measurements: The radii of curvature of the apical portion of the keratoconus were measured. In order to do this accurately, the profile of the corneal curve of the mold was projected onto the screen of an Optical Comparator.‖ The optics of this machine permitted an enlargement of 25 times the apical curvature. The apical curvature was then easily traced for a permanent record. Tracings were made of the corneal curvature in both the 90- and the 180-degree meridian. The curves were compared with curves of known radii which were similarly projected on the comparator in order to duplicate the magnification. The results of this study are shown in Tables 7 and 8.

‖ Do-All Sheet Metal and Furnace Company, Chicago.

TABLE 7.—*Change in Corneal Curvature (Radius) After Wearing Fluidless Lenses*

Case	Attacks of Hydrops	Years with Fluidless Lenses	Corneal Meridian, Degrees	Radius of Curvature (Mm.) After Wearing Fluid Lens		Radius of Curvature (Mm.) After Wearing Fluidless Lenses	
				O. D.	O. S.	O. D.	O. S.
1	3	2	90	5.0	5.0	5.0	5.0
			180	4.5	4.5	4.5	4.5
2	3	2.5	90	5.0	5.5	5.5	6.0
			180	5.0	5.5	5.5	5.5
3	1	2.25	90	6.0	6.0	6.0	6.5
			180	6.0	6.0	6.0	6.5
4	2	2.0	90	5.5	5.5	6.0	6.0
			180	5.5	5.5	6.0	6.0
5	4	2.5	90	5.5	6.0	6.0	6.5
			180	5.5	6.0	6.0	6.0
6	0	2.0	90	5.5	5.5	5.5	5.5
			180	5.0	5.5	5.0	5.5
7	2	2.0	90	6.0	6.0	6.0	6.5
			180	6.0	6.0	6.0	6.5

A total of 12 molds were available. Six of these molds revealed no change in curvature in either meridian, whereas the remaining six showed quantitative changes. Four of those showing quantitative changes revealed a flattening of the corneal curve in both meridians, and the remaining two, flattening of one meridian. In no case was there an increase in the corneal curvature.

Conclusions cannot be drawn from so small a series of measurements. It is hoped that this procedure will be observed clinically by other workers in the field, who, it is believed, will confirm our impression that the corneal lens not only exerts pressure on the apex of the conical cornea but also produces changes resulting in flattening of the apical curve.

TABLE 8.—*Per Cent of Change in Corneal Curvature After Wearing Fluidless Lenses*

	Increase in Corneal Curvature	No Change in Curvature	Decrease in One Meridian	Decrease in Two Meridians	Total
No. of eyes	0	6	2	4	12
Per cent of eyes	0	50	16.6	33.3	100

5. *Comparative Study of Subjective Symptoms with Fluid and Fluidless Lenses.*—Questionnaires regarding the subjective symptoms were sent to all patients in this study. Among the 18 patients who had fluid lenses there was a uniform report of several symptoms while wearing the lenses: (1) burning, (2) severe pains, (3) photophobia, and (4) blurring and decrease in visual acuity. Not all patients complained to the same degree, but each complaint was unequivocal. Each of these patients further reported difficulty and discomfort in the fitting of these lenses. In contrast, the same group had few subjective symptoms concerning corneal lenses. This was in agreement with the remaining 11 patients, who had no experience with fluid lenses.

The chief complaint of all patients wearing the multicurve fluidless lens was an initial period of pain and burning during the fitting and early adjustment period. These complaints usually subsided or disappeared in two or three months. Such pain may be due to the pressure of the corneal lens on the cone, resulting in eventual flattening of the corneal curve.

6. *Wearability of Fluid* vs. *Fluidless Lenses.*—As will be noted from Table 9, the daily wearing time of all patients was significantly increased to the point that all but four at present are wearing their lenses from 12 to 24 hours a day. Because these patients rely on their lenses as their only means of vision, these data take on an added meaning.

TABLE 9.—*Wearing Time; Fluid* vs. *Fluidless Lenses*

	1-6 Hr.	7-10 Hr.	11-12 Hr.	12-24 Hr.	Total
Fluid lenses	8	1	4	5	18
Fluidless lenses	2	1	2	13	18

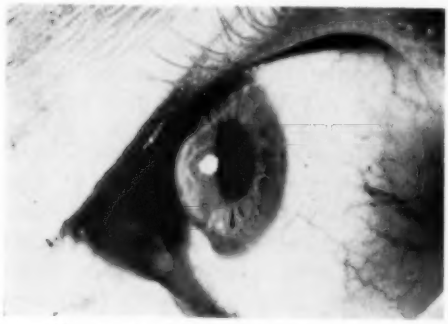


Fig. 5.—Corneal hydrops, lateral view.

TREATMENT OF CORNEAL HYDROPS IN PRESENT SERIES: PREVENTION OF HYDROPS

In most of our cases, the patient with hydrops was treated conservatively. He was not permitted to wear his fluid lenses, and pressure dressings were applied until the acute episode had subsided. In several instances, paracentesis was resorted to, in addition to the treatment mentioned. Several other methods of treatment for corneal hydrops have been described in the literature. Rohrschneider and Stohr [13] reported some success in cases of acute hydrops with the local administration of a hypertonic 40% dextrose solution. In addition, those observers refer to the method of Komoto, whereby an iridectomy is performed, followed by cauterization of the apex of the conus, and the method of Vogt, which consists of diathermizing the cone. We do not recommend these surgical procedures.

From our observations, it appears that the wearing of the corneal lens may in itself be a form of treatment for conical cornea or a preventive to the development of corneal hydrops. The corneal lens actually exerts pressure on the cornea; moreover, artificial fluids are not necessary when the corneal lens is used. Artificial fluids undoubtedly are contributory in the etiology of corneal hydrops through absorption into the corneal stroma with resultant edema and visual loss.

The entire subject of corneal hydrops (Figs. 5 and 6) through absorption into the corneal stroma, its pathogenesis and treatment, will be considered in a subsequent report.

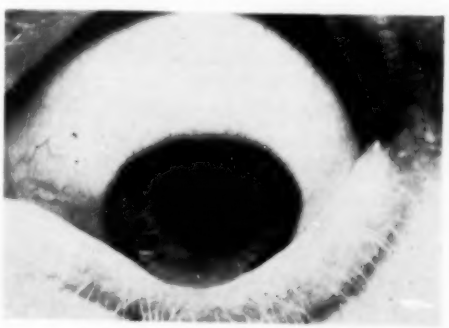
COMMENT

The use of fluidless corneal contact lenses in patients with keratoconus has been controversial for many years. The advisability of fitting a patient with a lens that actually comes in contact with the anterior corneal surface has been questioned. Hall [14] concluded from his experience that keratoconus patients should be fitted with lenses of the fluid type which completely clear the corneal apex. Unfortunately, his study was restricted to fluid lenses.

The results observed in our study are in greater agreement with those who have used fluidless lenses in keratoconus, as reported by Nugent,[11] Berens,[12] and others. The fluid lens fitted either with complete corneal clearance or with a central corneal touch is not indicated, in our opinion, in patients with keratoconus. Several significant observations lead to this conclusion: (1) The fluid lens apparently contributes importantly to the production of corneal hydrops through absorption of the artificial fluid into the cornea; (2) the visual acuity is diminished, and the subjective symptoms of patients, as shown in this series, are uniformly greater; (3) the fitting of fluid lenses in keratoconus patients is more difficult and the patient's daily wearing time is shorter than with fluidless lenses.

The introduction of a new type of fluidless contact lens with multiple inside curves now makes it possible to fit many patients with severe keratoconus who were heretofore unable to wear fluidless lenses. This is demonstrated in the present series of 29 patients,

Fig. 6.—Corneal hydrops, superior view, in same case as that in Figure 5.



who almost uniformly have experienced freedom from subjective symptoms. The incidence of corneal hydrops has been lowered. Visual acuity has been significantly increased.

SUMMARY AND CONCLUSIONS

The literature is reviewed for the purpose of disclosing advances in the optical correction of keratoconus which parallel the development of contact lenses, because heretofore the reports on keratoconus have been submerged in the reports on contact lenses.

A new fluidless corneal lens is introduced having multiple inside curves designed for use in severe cases of keratoconus in which the radius of curvature is greater than that found in single-curve lenses.

Our study comprises 29 patients classified as having medium to severe keratoconus. The patients have corneal curvatures with radii greater than 7.75 mm., i. e., a cornea which is more acutely curved than normal; therefore, these patients could not be successfully fitted with the conventional fluidless contact lenses. Of these patients, 60% have been observed for more than 10 years and 80% wore fluid lenses prior to the fluidless contact lenses now being worn.

Results of this limited study are highly favorable toward recommendation of the new lens described. Patients experienced subjective improvement, and measurable evidence is provided in terms of (*a*) increased visual acuity, (*b*) lowered incidence of corneal hydrops, (*c*) flattening of the corneal curvature, and (*d*) increased wearing time.

The use of these new multicurve fluidless corneal lenses is discussed with reference to the controversy whether a contact lens should actually come in contact with the apex of a corneal cone. The result of our study indicates that fluidless contact lenses *should* touch the corneal apex, and that fluid lenses are not indicated in the optical correction of keratoconus; indeed, they are contraindicated for patients with keratoconus because they may cause (*a*) progression of the cone, (*b*) increase in corneal vascularization, (*c*) increased irritability of the eyes, and (*d*) increased incidence of acute and chronic hydrops of the cornea with resultant corneal scarring.

It is proposed that the wearing of a multicurve fluidless corneal contact lens is actually a method of treatment for reduction in the curvature of the corneal apex of a patient with keratoconus.

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Effects of High-Energy Particles, X-Rays, and Aging on Lens Epithelium

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In this paper some evidence will be presented which bears upon three aspects of the problem of radiation-induced cataracts. One phase of the work pertains to the location of the radiosensitive epithelial cells, the damage of which might be the main causative factor in the formation of radiation cataract. The second part is concerned with the question of the so-called relative biological effectiveness of radiations with different linear energy transfers. A third section deals with the morphology of cell damage in the lens epithelium caused by the various radiations and that produced by old age.

The study of the first question will add to the understanding of the pathogenesis of radiation cataract. The second problem is important because presently known data indicate that heavily ionizing radiations, like neutrons, apparently have a greater effect in producing cataractous changes than lightly ionizing radiations, such as x-rays. Finally, comparison of the cytological changes produced by ionizing radiations and those occurring in old age may throw some light on the cataract problem in a wider sense. The experiments were conducted at the Donner Laboratory of Biophysics and Medical Physics and the Radiation Laboratory of the University of California, as well as the Laboratory of Radiological Research and the Department of Ophthalmology of Columbia University.

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From the Donner Laboratory of Biophysics and Medical Physics; the Radiation Laboratory of the University of California; the Radiological Research Laboratory, the Department of Ophthalmology, Columbia University College of Physicians and Surgeons, and the Institute of Ophthalomolgy, Presbyterian Hospital.

EXPERIMENTAL APPROACH

The principles and the advantages of using high-energy-charged particles for radiobiological studies were reported by Tobias, Anger, and Lawrence.[1] These authors described in detail the experimental set-up, including the treatment table for small animals controlled from a remote room, and gave an account of the physical properties of the approximately monoenergetic beam. The use of heavy particles in the experimental study of cataract induction has a twofold advantage: First, these particles may be directed with considerable accuracy through an aperture to any desired part of the eye. Since scattering is relatively small, neighboring structures, not directly in the path of the beam, will not be materially affected. The second advantage lies in the physical properties of deuterons and alpha rays. By suitable choice of absorbers, it is possible to regulate the rate of energy loss, or "linear energy transfer" of the particles, so that their biological effects would be similar to those of x-rays, on one hand, and to those of neutrons, on the other. An account of this type of study was given by

Zirkle and Tobias.[7] The evaluation of the radiation effects dealt with in this work is based on data of several cytological and histological studies undertaken on x-ray-induced lens damage at Columbia University.*

METHODS AND MATERIALS

As far as the use of high-energy particles is concerned, each irradiation involved an exposure to the beam of alpha particles or deuterons which passed through a shape-controlling aperture and stopped at a predetermined depth in an animal's eye. Dr. G. Failla, of Columbia University, designed a versatile holder for the rabbit which permitted alignment of the eye of the anesthetized animal with the beam. A schematic representation of this holder is shown in Figure 1. To allow precise alignment of pertinent landmarks in the eyes of individual animals with the center axis of the beam, plastic contact lenses were ground in three sizes, with a curvature radius of the scleral part measuring approximately 8.7, 7.9, and 7.1 mm. (Fig. 2). These lenses had a flat surface of 20 mm. A small volume of petrolatum U. S. P. (Vaseline) was placed in the concavity of the plastic cups before they were inserted in the anesthetized conjunctival sac. With this arrangement, a medium similar in density to that of tissue was interposed between the front absorber and the interior of the eye. After exposure to the beam the fit between the flat part of the contact lens and the protruding cylinder of the absorber (Fig. 3), which also

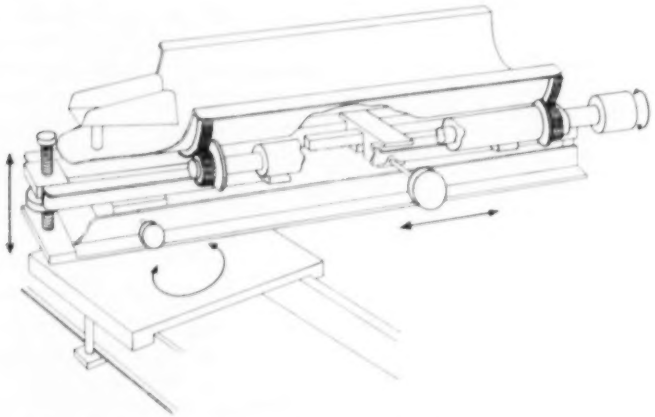


Fig. 1.—Schematic drawing of the animal holder designed by Dr. G. Failla. The anesthetized rabbits are placed on the reversible tray (top), which has a separate headrest. The entire apparatus is clamped onto the track of an optical bench, used for alignment of the beam. Separate adjustments are provided for rotation of the holder around a vertical axis, for raising or lowering, horizontal displacement, and angular displacement around the body axis of the animals. These adjustments are needed to allow exact placement of the eye prior to irradiation.

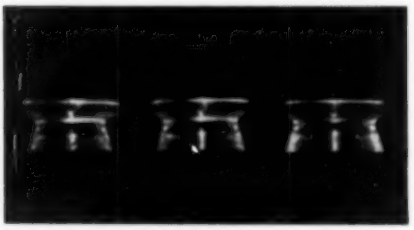


Fig. 2.—Plastic contact lenses.

measured 20 mm. in diameter, was checked for possible displacement. In the event that such displacement exceeded 2 mm., the experiment was not used for the quantitative evaluation of the results. Any rotation of the eye underneath the contact lens during exposure was noted, but disturbances of the geometry caused by such positional changes of the globe did not preclude the use of the experimental data in the individual series.

The 380 mev alpha particle beam or the 190 mev deuteron beam was first aligned by means of two quadrant ion chambers so that it traveled exactly parallel to the irradiation bench and 8 in. above its rails. The beam was fairly homogeneous over a circular cross-sectional area of 1½ in. diameter. It was passed through a monitoring ionization chamber of the parallel plate type, which continually measured the ionization over the central portion of the beam ½ in. in diameter. Concentric

* References 3, 4, 5, and 9.

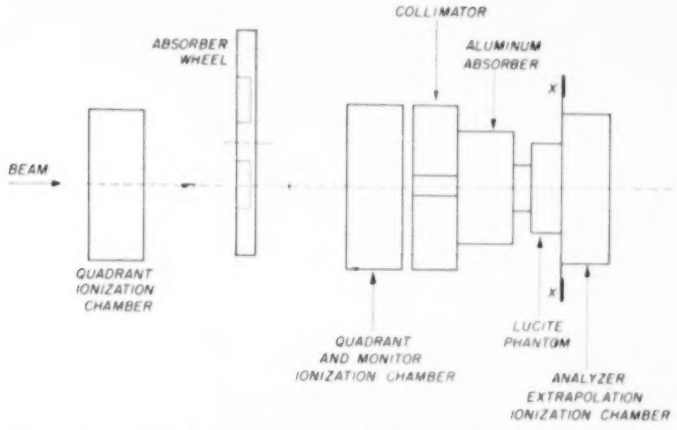


Fig. 3.—Schematic drawing of arrangement for measuring depth ionization curves in a phantom. The beam first passes through two thin quadrant ionization chambers to insure centering; it is then monitored, and it passes through a collimator of proper size, an aluminum absorber, a Lucite phantom, and, finally, a second "analyzer" ion chamber. The ratio of the analyzer and monitor-ion-chamber readings gives the relative ionization. An absorber wheel is also placed in the beam for small, remotely controlled changes in absorber thickness. The Lucite phantom has a calculated stopping power equivalent to the contact lens and the anterior segment of rabbit eye to the median plane of the lens.

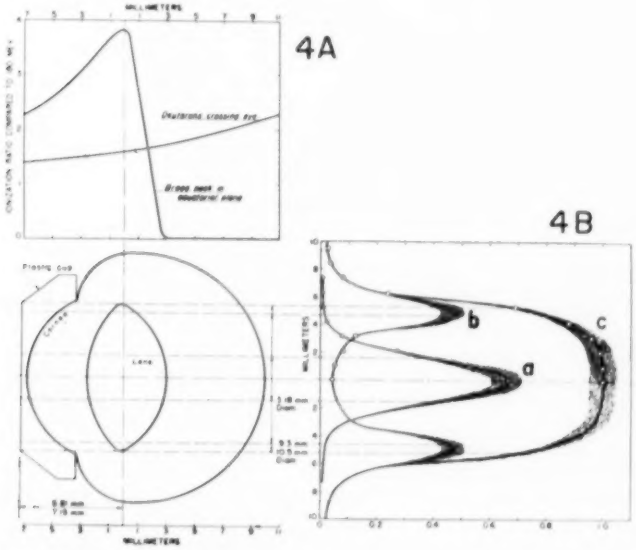


Fig. 4.—The dimensions of the rabbit eye are shown. In *4A* the variation of ionization is plotted on the same scale for high-energy deuterons and for the case in which the deuteron Bragg peak is adjusted to the equatorial plane of the lens.

4B shows the variation of ionization of the high-energy deuteron beam across the rabbit eye. Here, *a* is the intensity distribution after the beam passed through the 3.18 mm. aperture; *b* is the distribution due to the ring aperture, and *c* is the distribution due to the 10.5 mm. aperture. Relative ionization current is plotted on the abscissa with the ionization in front of the aperture 1.0.

with the collector plate of the ionization chamber, and immediately adjacent to it, a brass aperture followed, which limited the size and shape of the beam utilized. The beam then passed through an appropriate absorber of a wheel containing absorbers of various thickness and, finally, through a fixed front absorber before penetrating the plastic contact lens and entering the eye itself. Central round apertures of 3.18 and 20 mm. diameters, as well as a ring aperture with an inner diameter of 9.3 mm. and an outer diameter of 10.5 mm., were used. Attempts were made to achieve precise exposure by using the smaller central aperture for the central area of the lens and the annular aperture for the equatorial zone. In the early experiments the absorber selected stopped the beam behind the eye. In the main series, with a thick absorber, the peak of the Bragg curve fell approximately on the equatorial plane of the lens. This area is assumed to be the most vulnerable to radiation damage. In order to determine the dose and the dose distribution in the rabbit lens, the following steps were taken:

Variation of the total ionization over the area of the rabbit lens was measured behind the absorber by the arrangement illustrated in Figure 3. Here the rabbit eye was replaced by a "phantom" of Lucite, which had an absorbing thickness equivalent to the anterior portion of the eye from the apex of the cornea to the equatorial plane of the lens plus the contact lens. An analyzer ionization chamber was placed behind the phantom, and the ratio of the ionization between the front, monitoring chamber and the rear, analyzing chamber was determined. Figure 4*A* shows the variation in ionization as a function of the depth in the ocular structures with the high-energy deuteron exposure and the Bragg ionization curve maximum. In Figure 4*B* is shown the distribution of ionization of the beam across the rabbit eye when the various apertures were used. In order to determine the lateral distribution of the radiation, small cadmium sulfide crystals were employed.† The electrical conductvity of these crystals changes profoundly when they are subjected to ionizing radiation. The change in conductivity is so great that the initial number of radiation-produced ion pairs may be amplified by the current flowing within the crystal by a factor as high as 10^6. For the purpose of studying isodose surfaces, a small crystal of approximately $0.25 \times 0.25 \times 1$ mm. dimensions was employed in the present experiment. This was placed in the deuteron beam so that its long axis was parallel to the beam. The crystal was so sensitive that if 135-volt potentials were applied to it, 1 μam (microampere) current was

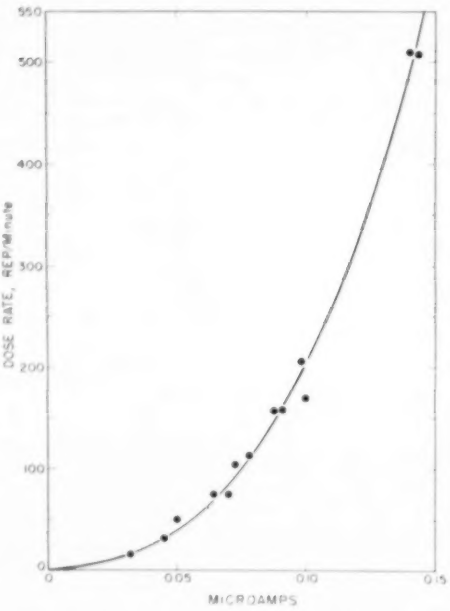
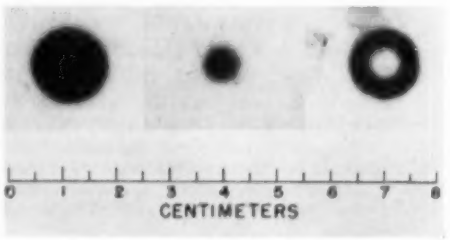


Fig. 5.—Calibration curve of one of the cadmium sulfide crystals for deuterons. The relation of the current passing through the crystals to the dose rate in the deuteron beam is shown. The dimensions of the crystal are $0.25 \times 0.25 \times 1$ mm.; 135 volt potential was applied.

obtained through the crystal with a dose rate of approximately 100 r per minute. The calibration curve is shown in Figure 5. By means of remote control this tiny crystal could be moved across the beam; as a rule, a reading was taken at every $\frac{1}{24}$ in., thus allowing adequate determination of the dose distribution. Since the response of the crystal is not very fast, the procedure was timed in such a way that readings were taken one minute after exposure and the crystal allowed to cool two

Fig. 6.—Radioautographs of the deuteron beam on an x-ray film placed in the phantom in a position corresponding to the equatorial plane of the lens.
Left: aperture diameter 10.5 mm.
Center: aperture diameter 3.18 mm.
Right: ring aperture.



† By courtesy of the General Electric X-Ray Company.

to six minutes before the next exposure. Because of the fluctuations in the over-all beam intensity, absolute readings were not made. Instead, simultaneous readings of the monitor chamber and the cadmium sulfide crystals were taken; the ratio of the two should then give the proper dose distribution of the beam. Figure 4*B* represents a typical beam distribution across the equatorial plane of the rabbit eye as measured in the phantom. In order to obtain more graphic demonstration of the dose distribution, x-ray films were exposed to the beam of deuterons under experimental conditions identical to those under which rabbit eyes with irradiated, with the plastic eye substituted for the intact eye. X-ray film is a nonlinear-radiation-recording device and cannot be used for dosimetry; nevertheless, the reproductions show fairly good delineation of the apertures. Such reproductions are shown in Figure 6.

From the above-mentioned calibration graphs it is evident that the dose varies from point to point over the entire volume of the lens or the eye. However, for the purpose of simplification the figures given in the dose table refer to the particular point considered most significant for the biological effects. For the large aperture, of 20 mm. diameter, the dose given was measured at the equatorial zone of the lens. For the small aperture the dose listed represents the maximum value at the center of the lens. For the annular-ring aperture the values listed indicate the maximum dose approximately in the middle of the ring, 5 mm. from the central axis of the beam.

In the actual animal exposures the animal's eye replaced the analyzer chamber and Lucite phantom, as shown in Figures 7 and 8. The monitor chamber was left in place and served as a standard-dose-monitoring instrument. The doses reported in this paper are in energy units (rep) at the median plane of the lens, computed from the phantom measurements and the monitor-ion-chamber reading. The procedure for dose calculation was previously described by Tobias, Anger, and Lawrence.

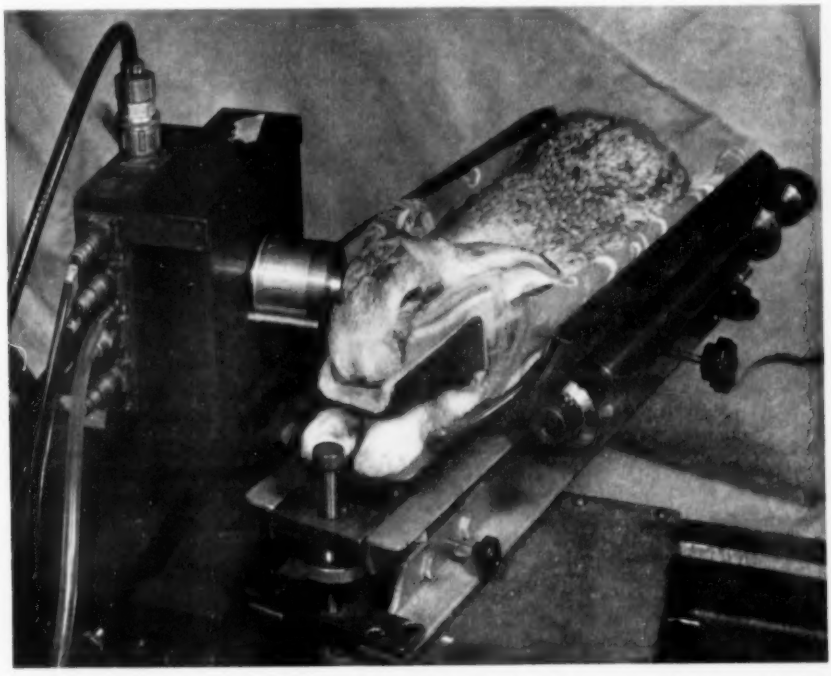


Fig. 7.—An anesthetized rabbit in position for irradiation: The contact lens over his right eye is in direct contact with the aluminum absorbers; the deuteron or alpha particle beam projects from the left, first passing through the monitor quadrant ion chamber, and then through the brass aperture, absorber, and eye.

The procedure of using phantoms for the determination of the dose rate in such biological experiments introduces a number of possible sources of error. First, the calculations are based on the assumption that the densities of the cornea, the aqueous humor, and the crystalline lens are equiva-

lent to that of Lucite (density = 1.15), whereas they differ slightly from this value. Second, there were some variations in the distance of the equatorial plane of the lens from the corneal apex among individual rabbits. This difference, however, did not amount to more than 0.3 mm. Third, during irradiation the energy of the beam brought out of the cyclotron may shift slightly. Such changes usually do not exceed 0.5 mm. in the region of the Bragg curve maximum. Finally, as mentioned previously, disturbances of perfect alignment of the axis of the eye with the beam might result from minimal movement of the head or rotation of the eyeball during exposure. The relatively great statistical fluctuations in the data may in some measure be due to the above-enumerated uncertainties, as well as to statistical fluctuations.

In the evaluation of data, it is important to know the actual value of the linear energy transfer for the insertion of a plastic contact lens, as used in the experiments with the heavy particles. The radiation factors were as follows: 210 kv.; 15 ma.; for doses of 1000 and 2000 r, TSD 17.5 cm., 590 r/min. in air; for doses of 125, 250, and 500 r, TSD 30 cm., 202 r/min. in air; 20 mm. field (round); 0.5 mm. Cu and 1 mm. Al filtration; HVL about 1.3 mm. Cu.

In all experiments the right eye of the rabbit was exposed to the beam of ionizing radiations; the left eye was outside the beam and in the x-ray series was shielded to serve as control. For studying the effects of high-energy alpha particles and deuterons and, in a comparative series, of 210-kv-produced x-rays on cell division and on early injury to the cell nucleus, the animals were killed with an overdose of pentobarbital sodium as a rule 2, 4, 6, 10, 14, or 21 days after treatment. In the experiments in which 125 and 250 rep was

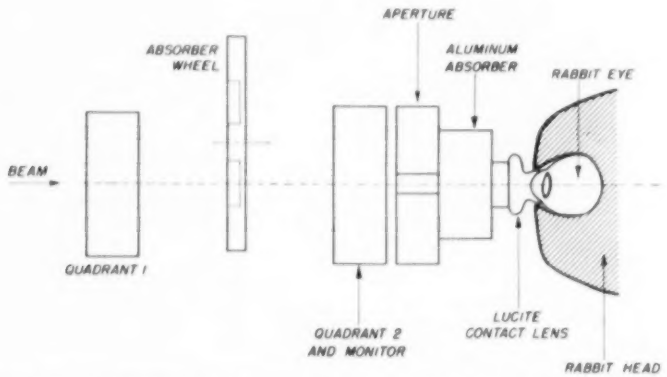


Fig. 8.—Schematic drawing of the actual exposures. The animal's head replaces the phantom of Figure 3. Care was taken to align the eye with the center of the beam and to have the Lucite contact lens in physical contact with the absorber.

TABLE 1.—*Mean Values of Linear Energy Transfer (LET) for X-Rays, Deuterons, and Alpha Particles*

Radiation	Average Value of LET
200 kv. x-rays	3 kev/μ
Deuterons, peak of Bragg curve	4 kev/μ
Helium ions, peak of Bragg curve	23 kev/μ

(LET). Owing to multiple scattering and straggling, the particles are not monoenergetic, but show a considerable spread of linear energy transfers. Table 1 shows the mean values assumed for the different radiations.

The technique of exposure to the x-ray beam was similar to that described in previous publications from the Columbia laboratories‡ except administered, groups of animals were killed also at 6 and 24 hours after exposure to each of the three radiations. In addition, x-ray-treated rabbits were killed at 10-minute intervals within the first hour after irradiation.

The x-ray studies were conducted exclusively on male chinchilla rabbits approximately 2 to 3 months of age. In the experiments with the high-energy particles various breeds of male rabbits of a similar age were used. The quantitative results of radiation-induced interference with cell division and of degenerate cell counts were obtained by cytological examination of approximately 500 epithelial preparations of irradiated lenses and the same number of controls. The lenses examined for qualitative radiation effect were far in excess of this figure. Age-dependent cell degeneration in rat lenses supplemented the search of quality effects of ionizing radiations on the lens epithelium. The

‡ References 3 to 5.

methods used in this part of the study will be given, together with the results.

The technique of the histological and cytological examination has been reported previously. The Feulgen reaction in whole mounts of the lens epithelium was carried out with minor modifications according to the description of Howard.[6] In a small number of experiments the lenses, embedded in paraffin and meridionally sectioned, were stained with hematoxylin and eosin or according to the Feulgen technique. Prolonged observations of experimental animals for development of lenticular opacities were of necessity limited to a small group.

preequatorial and equatorial part of the epithelium than in the central portion. The nuclei of the lens bow do exhibit positional, but no morphological, changes within three weeks after irradiation with doses up to 2000 rep.

This conspicuous difference in radiosensitivity of the respective parts of the epithelium was confirmed in 40 experiments in which a central area or a peripheral ring zone of the lens of one eye was subjected predominantly to the action of the relatively

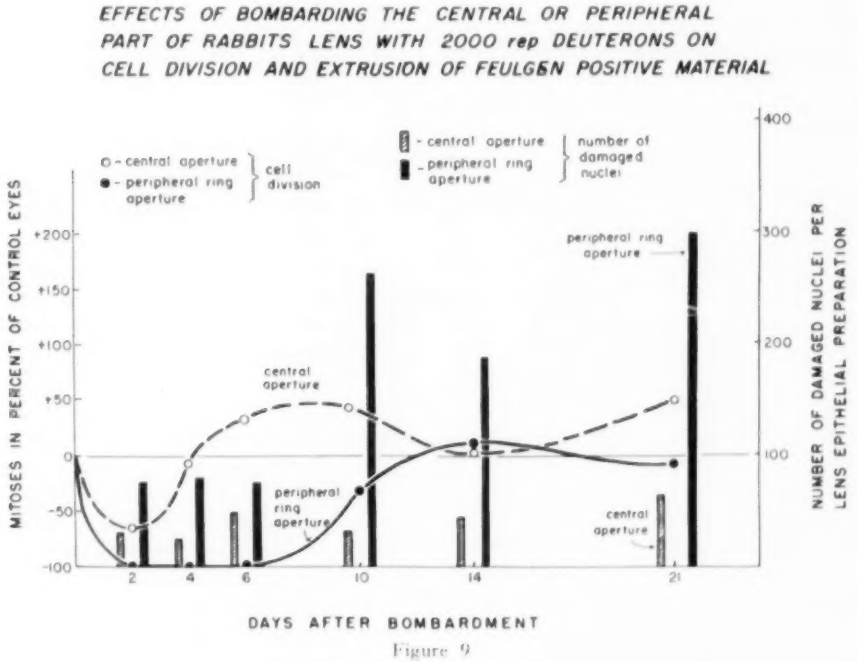


Figure 9

RADIOSENSITIVITY OF RABBIT LENS EPITHELIUM AT CENTRAL AREA AND PERIPHERAL ZONE

When a beam of ionizing radiation at sufficient dose level strikes the rabbit lens, the extent of cell damage in the germinative zone surpasses by far that in the central area; likewise, the radiation-induced depression of mitotic activity and the subsequent overcompensation of this function, with the accompanying appearance of pathologic mitoses, are overwhelmingly greater in the nonscattering deuteron beams. The composite graph of Figure 9 illustrates the average effects on cell division, in percent of the counts of the control eye, and the occurrence of damage to cell nuclei when 2000-rep deuterons were administered through a 3.5 mm. central opening or through the ring aperture, the dimensions of which have been stated in the technique. The average counts are plotted against time at which the experiments were terminated. Bombardment of the equatorial zone was followed by almost complete depres-

sion of mitotic activity for six days, a result similar to that observed after irradiation of the lens with 20-mm., full-beam deuterons of the same dose level. However, the rise of mitotic activity to supernormal levels subsequent to the inhibition was absent, whereas it regularly occurred when the entire lens was irradiated. In the experiments with the small central aperture the diminution of mitosis was limited to about 50% of the number of dividing cells in the control eyes; it was of short duration and was succeeded by an increase of cell division for about the same percentage value. The average ratio of damaged cell nuclei resulting from the irradiation of the germinative zone versus the central area was 4.3:1 for all time intervals, but no great significance can be attributed to this figure for the following reasons: Observations on position of the corneal apex relative to the center of the flat part of the contact lens prior to and after the bombardment showed that in many instances rolling of the eye or movements of the head for 1 to 2 mm. had resulted in a shift in the alignment of the system during the exposure. Results of cytological examination of these eyes indicated a definite influence of this slight decentration of the beam during a part of the irradiation. Thus, in such experiments with the small central aperture, injury to cell nuclei occurred frequently in one sector of the germinative zone, which also exhibited corresponding radiation effects on cell division, or in experiments with the ring aperture the changes prevailed in one part of the preparation. Slight disturbances of alignment, then, tend to lessen the contrast in radiosensitivity of the cells in the two areas and strengthen the over-all evidence of the low vulnerability to ionizing radiations of cells in the large central part of the epithelium as compared with the high sensitivity of the cell elements in the narrow germinative zone.

Only a few rabbits which had been irradiated through the small central aperture or the ring aperture were observed for prolonged periods. The ring aperture was used in four experiments with deuterons for two dose ranges, 180 to 240 rep and 2100 to 2200 rep. Granular opacities were noticed in the posterior cortex of lenses irradiated with the lower dose six to four months after treatment. Bombardment at the 2100 rep dose produced initial lenticular opacities within three to five weeks and total cataract in four months. Only one of the animals which had been treated with a dose of 2000 rep through the small central aperture survived sufficiently long for evaluation of late effects. Nine months after bombardment the lens appeared to be clear; at this time a severe ear infection which had caused death of other animals of this group at earlier periods necessitated termination of the experiment.

COMPARISON OF EFFECTS OF 210-KV-PRODUCED HIGH-ENERGY ALPHA PARTICLES, AND DEUTERONS ON MITOSIS AND CELL DEGENERATION

Effects of Irradiation with X-Rays

The 208 experiments with x-rays include a series of 36 rabbits in which the radiation effects on cell division were studied at intervals of 10 minutes for one hour after exposure to 1500 r. The phase counts of this group and of a control series of 52 eyes are contained in Tables 2 and 3, and Figure 10 illustrates graphically the averages for various irradiated and control eyes at various time intervals. Prophase counts already appeared decreased at the 10-minute interval, whereas telophase counts were affected last, with complete absence of dividing cells in the entire population about one hour after exposure. Since the animals were anesthetized with sodium pentobarbital during irradiation and stickiness effects on chromosomes were noted, the apparent duration of the mitotic phases should not be considered to give the accurate timing of the undisturbed function.

The main series of experiments with x-rays comprises data obtained with five radiation doses (125, 250, 500, 1000, and 2000 r) at six time intervals, i. e., 2, 4, 6, 10, 14, and 21 days after exposure. In addition, counts obtained at 6 and 24 hours provided information on effects for the two smallest doses of the three types of radia-

TABLE 2.—*Mitotic Phase Counts in Rabbit Lens Epithelium Within Fifty Minutes After Irradiation with 1500-r X-Rays*

Minutes After Irradiation	Prophase			Metaphase			Anaphase and Telophase		
	No. of Preps.	Counts, in %, of Control Eyes (Average)	S. D.	No. of Preps.	Counts, in %, of Control Eyes (Average)	S. D.	No. of Preps.	Counts, in %, of Control Eyes (Average)	S. D.
10	6	— 77	11.3	6	—15	9.9	6	— 5	26.7
20	7	— 85	13.3	7	—43	33.1	7	— 2	37.9
30	8	— 99	1.4	8	—74	8.4	8	—35	19.6
40	8	—100	0	8	—92	3.8	8	—58	6.48
50	7	—100	0	7	—90	2.2	7	—88	5.19

TABLE 3.—*Mitotic Phase Counts in Lens Epithelium of Normal Adult Rabbits*
(52 Preparations)

Prophase		Metaphase		Anaphase and Telophase	
Counts, in %, of Control Eyes	S. D.	Counts, in %, of Control Eyes	S. D.	Counts, in %, of Control Eyes	S. D.
38.67	21.7	58.3	21.8	33.96	20.2

tions, although possible disturbances by the systemic anesthesia somewhat hinders evaluation of the early results.§

§ The individual counts are not reproduced in this paper because of their great number, but tabulations of these data are available to those who request detailed information.

In the series 172 rabbits were used, and 3 to 10 observations yielded averages for each dose and for each time interval in the three-week period. Irradiation with 125 r initiated a questionable delay of mitosis at the six-hour interval; at 24 hours the average inhibition of cell division amounted to 78% of the counts in the control eye. At this time the radiation effect was maximal for this dose. In the experiments with the 250 r dose the diminution of mitosis was moderate but definite six hours after irradiation (—18%), and again at maximum at 24 hours (—82%). From here the mitotic counts rose in both groups and reached normal ranges after four and six days for

MITOTIC PHASE COUNTS IN RABBIT LENS EPITHELIUM WITHIN 50 MINUTES AFTER IRRADIATION WITH 1500r X-RAYS

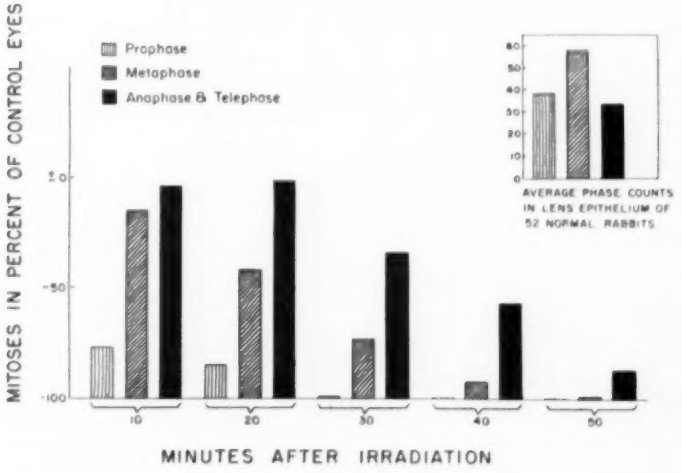


Figure 10

EFFECT OF X-RAYS ON MITOSIS IN RABBITS LENS EPITHELIUM

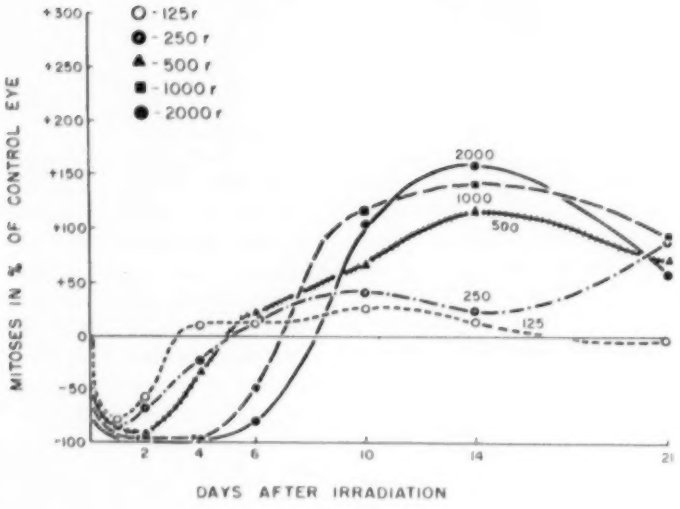


Figure 11

RADIATION INDUCED DAMAGE TO CELL NUCLEI IN RABBITS LENS EPITHELIUM

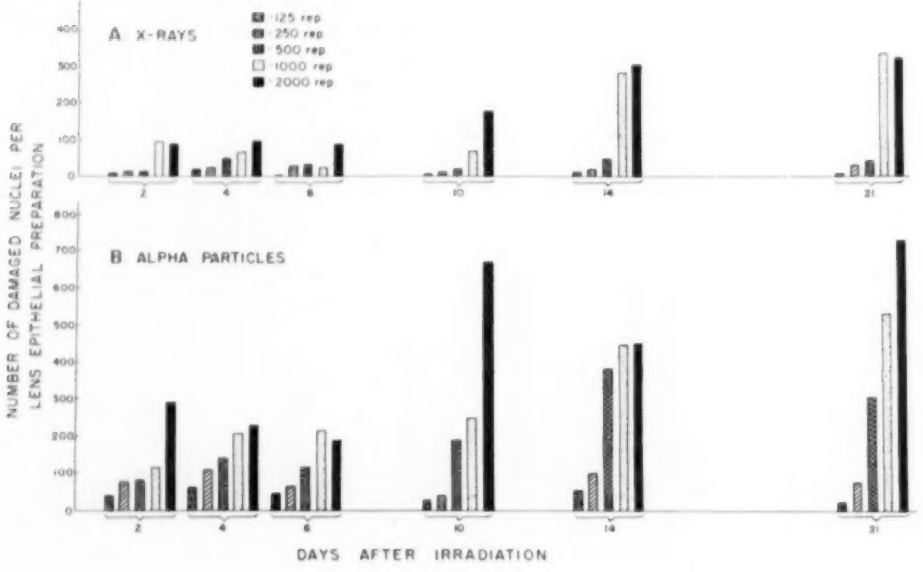


Figure 12

the 125 and 250 r groups, respectively. Peaks of the following overshoot were recorded at 10 days for both doses. Inhibition of cell division was complete for two days when 500 r was used. The delay of mitosis lasted four days when 1000 r was administered, and almost six days after a dose of 2000 r. In the latter case a small number of dividing cells were seen toward the end of this period. The successive overcompensating increase of cell division reached a maximum at 14 days after irradiation with doses of 500, 1000, and 2000 r. In general, the number of dividing cells, counted at the individual periods, showed rough proportionality to the dose up to a level of 1000 r, as seen in Figure 11.

At the earliest intervals degenerate cells were encountered infrequently in the experimental eyes of the 125 r series and, at six hours, in three of the six preparations in the 250 r group. At 24 hours after exposure to the latter dose four of the five mounts showed such cells, with an average of 6.6 per preparation. Dose dependency of this early biological effect was more marked when the results of all five doses were compared.

The incidence of degenerated nuclei per lens preparation rose progressively with the dose up to a level of 1000 r (Fig. 12*A* ‖). One exception to this rule was noted at the six-day interval, when the average of the counts on lenses exposed to 1000 r was unexplainably low. The extent of the cell damage increased conspicuously with time during the three weeks of observation in the 1000 and 2000 r series, whereas such an increase was moderate or absent in the low-dose experiments.

Effects of Bombardment with Alpha Particles

The description of the delay of cell division which was given for the experiments with x-rays can be applied with modifications to the results of the series of 157 experiments with alpha particles. The initial delay at six hours induced by a dose of 125 rep was of the same magnitude as that in the x-ray series, but the mitotic counts decreased 24 hours after bombardment by only 50% of those in the control eyes and did not seem to change for another 24 hours before rising to normal levels. The response to a dose of 250 rep appeared slower than that to x-rays, since at 24 hours the depression amounted to 65.4% and dropped to 82.5% of the counts in the control eye only after a lapse of 48 hours. For the higher-dose ranges the temporal sequence of diminutions of mitotic activity resembled that in the x-ray series (Fig. 13). Complete inhibition of cell division lasted, depending on the dose, from two to six days before recovery began. At the two-week interval, however, the overcompensating increase of cell division for all doses reached values about twice as high as those observed in the x-ray group. Increments of 200% to 300% in the numbers of dividing cells above those in the control eyes were recorded at higher doses.

The number of cells which showed extrusion of chromatin from the nucleus or late stages of karyorrhexis was sizable already at the dose ranges of 125 and 250 rep (Fig. 12*B*). Degenerate cells were seen in three of four preparations at 6 hours (average 3.7 cells per preparation) and in all mounts at 24 hours (average 17.6) in the 125-rep series. The degenerate cell counts were 4.3 and 75.2 for the two earliest time intervals following bombardment with a dose of 250 rep. Peak levels of 600 and 700 injured cells were averaged in experiments in which 2000 rep had been used. Here the high incidence of degenerate cells exceeds the mitotic counts of normal rabbits by a factor of 3 to 4. With one exception, at the six-day interval, dependence of effect on dose was indicated at all periods. Continuous progression of the cell injury within the three weeks after irradiation was clearly evident but was not consistent in the 2000-rep group. Figure 12 contrasts the averaged

‖ Apparently, in doses higher than 1000 r saturation level of cell damage was approached and differences in radiobiological effects caused by the higher dose were not consistent.

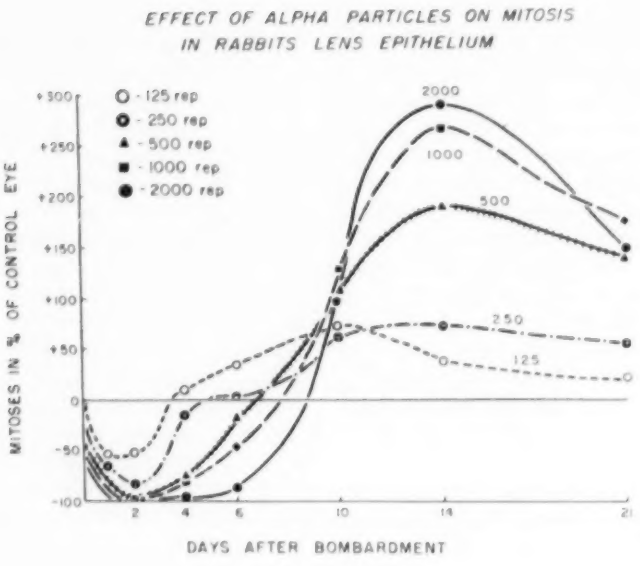


Figure 13

degenerate cell counts in the x-ray and alpha particle series. At this point it must be stressed again that there was a wide spread of the numbers of cells, with early signs of radiation injury among individual groups of identically irradiated animals which were killed at the same time after irradiation.

Effects of the Bombardment with Deuterons

The deuteron series comprised a relatively small number of experiments (109 animals), and individual groups of the high-dose ranges consisted several times of only two observations. In view of the great variations in the two described radiobiological effects in identically treated animals, the results are less reliable that those of the alpha particle and x-ray series. In general, timing and extent of delay and the subsequent overshoot of mitotic activities followed a course which resembled that described for the experiments with alpha particles. However, after irradiation with 125-rep deuterons mitotic counts declined to a minimum of 80.5% at the 48-hour interval, and thus exceeded the decrease which followed the exposure to alpha particles at this dose range. Such depression was also more marked at the 24-hour and 48-hour interval with the 250 rep dose. On the other side, the degenerate cell counts of lenses treated with alpha particles surpassed those of the deuteron group by a factor of 2 when the first time intervals alone were considered. No essential deviation of results in the deuteron series from those reported in the experiments with alpha particles was noted for the higher doses, either with respect to time sequence and dose dependence of effects on cell division or with regard to the severity of signs of cell injury.

Relative Biological Effectiveness of Alpha Particles and Deuterons Versus X-Rays for Rabbit Lens Epithelium

The data on degenerate cell counts and on mitotic counts were submitted for statistical analysis to Dr. J. E. Silson.¶ The procedure which was followed is outlined in the Addendum. The specific RBE, $\frac{\text{rep of x-rays produced by 210 kv}}{\text{rep of alpha particles (or deuterons)}}$, which refers to the weighted averages of degenerate cell counts at the given time intervals after

¶ Division of Biostatistics, School of Public Health of the Faculty of Medicine, Columbia University.

exposure to the five doses of radiations, was found to average 4.1 for alpha particles and varied from 2.7 to 6.1 when a 99% confidence range was selected; it was calculated as 3.9 for deuterons versus x-rays, or from 2.3 to 6.6 for the 99% confidence range. Attempts were made also to establish values for the RBE of deuterons versus alpha particles, but variations among individual counts in various groups and inconsistencies of small differences obtained between the two forms of radiations at individual time intervals precluded the possibility of arriving at a significant ratio for the two effects.

The contrast between the extent of early cell injury caused by the densely ionizing particles and that caused by x-rays was not apparent when suppression of cell division and of the overcompensating increase of the function was taken as the criterion of early radiation effects on a cellular level. No significant differences were noted in the mitotic counts for any but the 14th day, when the RBE of x-ray/alpha particles appeared quite large. It averaged at this time 2.46. This value varied from 1.5 to 4.1 with a 95% confidence range and from 1.1 to 5.3 for the 99% confidence range.

EFFECTS OF ALPHA PARTICLES, DEUTERONS, AND X-RAYS ON DISPLACEMENT OF NUCLEI FROM LENS BOW AND ON CELL COUNTS IN PREEQUATORIAL EPITHELIUM

Two other early signs of radiation damage, dislocation of nuclei from the lens bow and reduction of the number of cells in measured areas of the preequatorial zone, were compared in a small group of rabbits two weeks after bombardment of one eye with the densely ionizing radiations and after irradiation with x-rays. A dose of 2000 rep was applied. The paraffin technique described in previous studies[3] from the Columbia Laboratories was changed by omitting the removal of a calotte from the posterior cortex and the delivery of the lens nucleus prior to the embedding procedure. After fixation, experimental and control lenses were simply dissected in a meridional plane and the halves subjected to a shortened dehydration procedure. Five-micron sections were stained with hematoxylin and eosin or treated according to the Feulgen technique.

Displacement of the bow nuclei in the group in which alpha particles or deuterons had been used exceeded the extent of this change in the x-ray series in a 9:1 ratio at the two-week interval: The results of the individual experiments are shown in Table 4, where each figure is an average of counts for from 10 to 28 sections of the individual experimental and control lenses. Exposure to the high-energy beam resulted in the displacement of about 11 nuclei per section, as compared with about 1 nucleus in the x-ray group. The average decrease of the number of cells in measured areas of the preequatorial zone amounted to 20 per section in the eyes bombarded with the heavy particles and to about 5 in the x-ray-treated eyes.

TABLE 4.—*Number of Nuclei Displaced* from Bow of Rabbit Lenses After Irradiation*

Time After Irradiation, Days	Type of Radiation: 2000 rep Alpha Particles and Deuterons			Type of Radiation: 2000 r X Rays		
		O. D.	O. S.†		O. D.	O. S.†
7	1	1.5	0	..	....	....
	2	1.46	0	..	....	....
	3	1.85	0.03	..	....	....
	4	2.4	0	..	....	....
	Average 1.8					
14	5	7.37	0	8	0.46	0
	6	17.9	0	9	1.12	0.06
	7	7.2	0	10	1.98	0.12
	Average 10.8			Average 1.2		

* Each figure is the average of counts for 10-29 sections of each lens.
† O. S., nonirradiated controls.

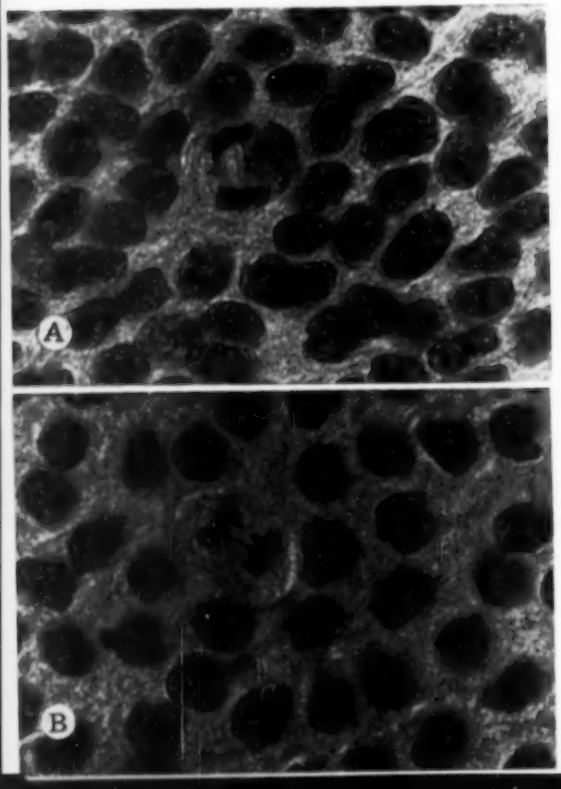
TABLE 5.—*Cell Counts* in Precquatorial Epithelium of Rabbit Lenses After Irradiation*

Time After Irradiation, Days	Type of Radiation: 500 rep Alpha Particles and Deuterons			2000 r X-Rays		
		O. D.	O. S.†		O. D.	O. S.†
7	1	87.7	90.4	..	....	....
	2	83.4	95.1	..	....	....
	3	95.6	90.0	..	....	....
	4	90.1	94.7	..	....	....
	Average diff. —4.6					
14	5	68.7	93.3	8	89.7	88.2
	6	72.7	84.4	9	84.5	92.2
	7	76.1	96.3	10	79.0	88.2
	Average diff. —20.3			Average diff. —5.2		

* Each figure is the average of counts for 10-29 sections of each lens.
† O. S., Nonirradiated controls.

Since the figures in the x-ray series were very low (Table 5), and close to the margin of error in the two-week period, no experiments were carried out one week after irradiation. The results in this small group of experiments point to differences in the quantitative effects of the two types of radiation which are in line with the observations on induction of cell degeneration in the main series.

Fig. 14.—Chromosome bridges in dividing cell of rabbit lens epithelium 20 minutes after irradiation.



MORPHOLOGY OF EARLY CHANGES OF LENS EPITHELIUM CELLS IN RABBIT EYES FOLLOWING EXPOSURE TO HIGH-ENERGY PARTICLES OR X-RAYS AND AGE-INDUCED CELL DEGENERATION IN THE RAT LENS

Of the 1379 rabbits which were utilized in the study, including those reserved for histological examination of paraffin-embedded lenses or for long-term observation, 1028 preparations (514 rabbits) were sufficiently intact to be subjected to mitotic and degenerate cell counts in whole mounts. For the examination of qualitative radiation effects on the lens epithelium, hundreds of flat preparations could be added which were excluded from the quantitative study because the mounts were grossly defective, the control of the paired preparations was lost by rupture of the lens capsule during the hydrolysis procedure, or, in early series, the dosimetry was not accurate. Thus, the material available for cytological evaluation exceeded by far the number of specimens in similar work on radiobiological effects. It permitted the examination for specific forms of early cell changes in this epithelium, that is, for quality differences in the injuries caused in the cell by the individual type of radiation under consideration.

The earliest morphological changes were observed in cells which had entered mitosis at the time of irradiation and were examined at 10-minute intervals during the first hour after exposure of the eye to 1500 r x-rays. Chromatin bridges, which are considered as caused by stickiness effects of irradiation,

were noted in anaphase and telophase of cells in mitosis 20 minutes after irradiation; this change implicated all remaining dividing cells at the 40- and 50-minute intervals (Fig. 14).

During the period of inhibition of cell division a varying number of cells showed globules of Feulgen-positive material outside the cell nucleus, accumulated either in the cytoplasm, and often indentating the nucleus, or in the intercellular spaces. The intensity of staining of the chromatin matter, the size of the globules, and their numbers varied greatly, but independently of the type of the three radiations studied. Later, in the intercellular space, "globules" of different sizes may become confluent or aggregate in clusters, which are frequently surrounded by nonstaining aureoles. Spear and Glücksmann [14] thought that these "aureoles" represent the nonchromatic part of the nucleus. The extrusion of chromatin material often results in complete fragmentation of the nucleus, after which the cell vanishes. In this case a characteristic gap appears in the regular pattern of the epithelium; it is temporarily occupied by an accumulation of round chromatin masses. At other times the injury to the cell nucleus is apparently of minor, and possibly of reversible, nature, but extrusion of Feulgen-positive material at a given time can represent various stages of the degenerative process. The regular arrangement of the normal epithelial cells with a few chromatin particles interspersed between them may indicate that their presence does not signify a radiation-induced lethal cell effect. Several days later a part of the clumped nuclear material had lost its stainability to a high degree or completely. A new crop of cells with the described degenerative changes or residues of such cell nuclei appeared at the period of excessive cell division which followed the period of depression. The number of damaged cell nuclei increased steeply at this time. In addition to the globular masses of chromatin, heaps of angular particles were then observed, which obviously developed from clumping and disintegration of chromosomes during cell division. These signs of radiation-induced cell damage also were qualitatively similar in all three series, in contrast to the great difference in the incidence of the lesions after exposure of the eye to lightly or densely ionizing radiations.

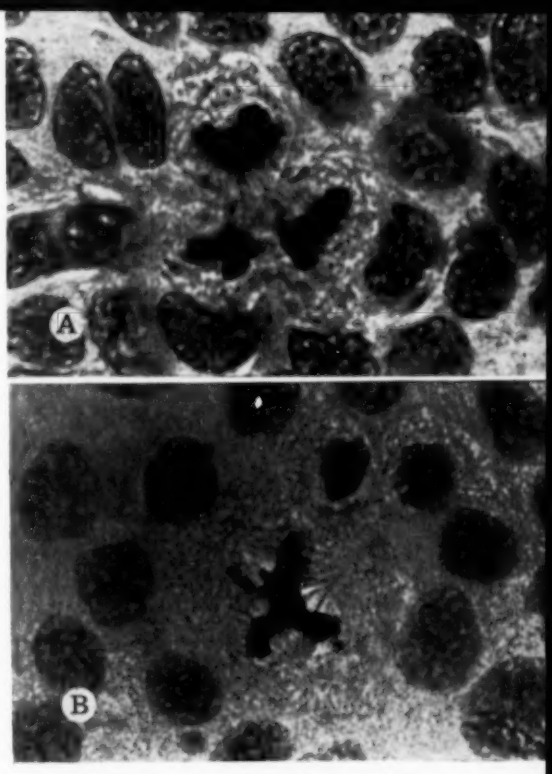


Fig. 15.—*A*, tripartite cell division in epithelium of irradiated rabbit lens. *B*, tripolar spindle in epithelium of irradiated rabbit lens.

Abnormal mitoses following the delay of cell division were manifold but resembled each other in the three experimental groups. However, one abnormal form of cell division, which consisted of multipolar dividing figures, was encountered most frequently in eyes which had been bombarded with alpha particles at varying dose levels. This change was noted in the later series, and, since it may have been overlooked in earlier experiments, no quantitative data on the occurrence of such abnormal mitoses can be offered. Tripartite spindles were seen in 12 preparations of this group. In the Feulgen-stained mounts the spindle fibers were not always visible with equal clarity, but this did not obviate the identification of such dividing cells. Usually the chromatin material formed a three-pronged figure, in the shape of a Y, which gave rise in later stages to forms with

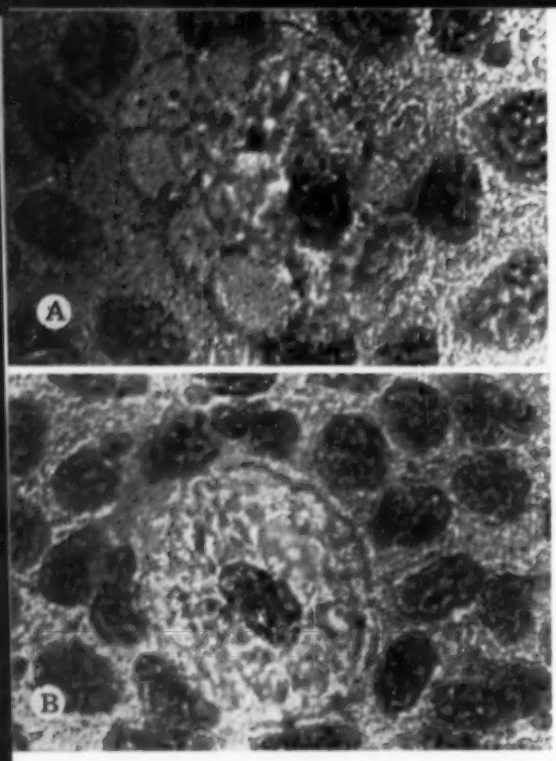


Fig. 16.—*A*, excessive vacuolar degeneration of cytoplasm with nuclear fragmentation in epithelium of irradiated rabbit lens. *B*, excessive vacuolar degeneration of cytoplasm in epithelium of irradiated rabbit lens.

three equal-sized portions of the chromatin in a regular arrangement (Fig. 15). Incidences of tetrapolar karyokinesis were also recorded. In subsequent examinations of the lens preparations from eyes treated with deuterons multipolar mitoses were encountered, and recently, as rare findings, a few tripartite and quatripartite figures were observed in eyes which had been exposed to x-rays. Excessive vacuolar degeneration of the cytoplasm was seen in the lens preparations after bombardment of the eyes with heavy particles (Fig. 16). Such a change was extremely rare in eyes examined within three weeks after x-irradiation with doses used in the study. It seems, then, that multipolar forms of cell division and excessive vacuolar degeneration of the cytoplasm signify a quantitative rather than a qualitative difference between effects of densely and lightly ionizing radiations on the lens epithelium.

In the supplementary study on age-induced changes of mitotic activity and cell degeneration in the lens epithelium, counts or morphologic analyses were made on 94 eyes of rats ranging in age from about 50 days to more than 1000 days. Dr. George Smelser supplied us with most of the lenses of animals in the 900-day+ age group; they were made available to him by the Wistar Institute from their geriatric colony many years ago. The Feulgen technique on flat mounts was used in the young and middle-age group. Since the eyes from the Wistar colony had been fixed in formalin immediately after the rats were killed and then stored in alcohol, flat preparations of the epithelium of these lenses were often unsatisfactory, and the Feulgen reaction could not be carried out on this material. However, iron-hematoxylin staining gave usually good results for morphological studies. Mitotic and degenerate cell

TABLE 6.—*Effect of Age on Mitosis in Rat Lens Epithelium*

Rat Age, Days	No. of Preparations	Averaged No. of Mitoses Per Preparation	Standard Deviation
40-50	37	106.2	25.7
—150	67	71.8	29.3
700-900	10	61.6	10.9
900-1,025	11	24.6	10.2

counts on relatively complete preparations were calculated in 11 of 16 lenses of the animals older than 900 days. The average counts of dividing cells decreased from 106.2 per preparation, in the 50-day-old animals, to 24.6, in the group which comprised lenses of animals older than 900 days (Table 6). In preparations obtained from old animals, differences in size and stainability of cell nuclei in all zones of the epithelium were prominent, and various types of cell degeneration increased in number with age. Particularly high was the incidence of micronuclei or accessory nuclei, which reached counts of 100 and more in preparations of the oldest group (Fig. 17). Pyknotic nuclei occurred also in considerable numbers. Extrusion of globular material from the nucleus (Fig. 18) and bizarre forms of nuclear fragmentation, giant nuclei (Fig.

19*A*), and cell division by budding were observed in many preparations. Multinuclear cells and a few multipolar dividing figures closely resembled those seen in later stages of radiation damage. Occasionally vacuoles occupied a central or paracentral area of the nucleus (Fig. 19*B*), a change noticed in other tissues as a radiation-induced damage.

Excessive vacuolar degeneration of the cytoplasm was present in several preparations of the old-age group (Fig. 20). This change was indistinguishable from that in relatively young rabbit lenses after exposure to ionizing radiations. With the material at hand it was impossible to relate extent of cell degeneration to the development of lenticular opacities which occur in old rats, but it is not unlikely that such cell changes precede cataract formation.

Long-Term Observations

At the time of bombardment of the rabbit eyes with high-energy particles it was planned to set aside three animals in the 1000- and 2000-rep series for determining the latency period between exposure and the appearance of lenticular opacities for each radiation. Unfortunately, the small number of animals reserved for this purpose was further reduced by accidental death. The results tabulated in Table 7 indicate the onset of biomicroscopically visible changes, vacuoles, or fine opacities, situated in various parts of the lens cortex. When the eyes were bombarded with 1000 rep of alpha particles or deuterons, such changes were detected after 3 to 12 weeks, and for the

Table 7.—*Onset of Cataractous Changes in Rabbit Lenses After Irradiation with Alpha Particles, Deuterons, or X-Rays*

Type of Radiation	Dose, in Rep	No. of Animals	Latent Period, Wk.
Alpha particles	1,000	3	4-12
	2,000	2	4
Deuterons	1,000	2	3-10
	2,000	3	2-5
X-Rays	1,000	8	21
	2,000	52	(4-6)*, 7-10

* Latent periods of four to six weeks were encountered in less than one-third of this group.

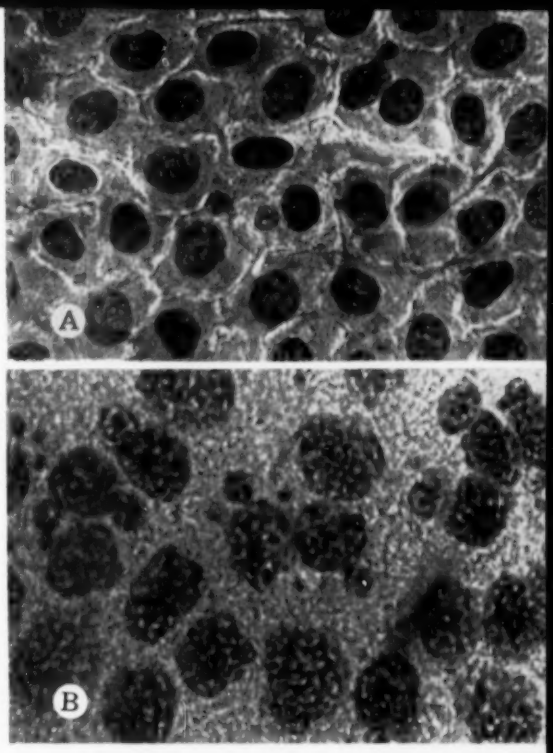
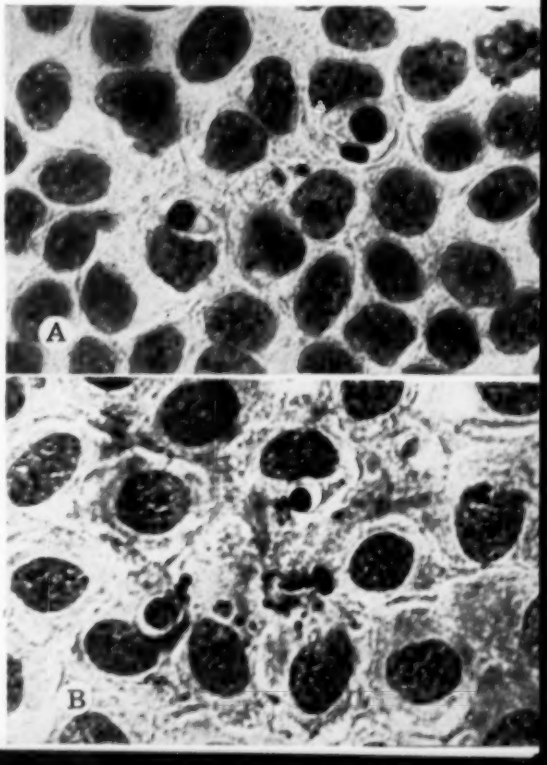


Fig. 17.—*A*, accessory nuclei in lens epithelium of old rat. *B*, accessory nuclei in epithelium of irradiated rabbit lens.

Fig. 18.—*A*, extrusion of chromatin material from cell nuclei in epithelium of irradiated rabbit lens. *B*, extrusion of chromatin material from cell nuclei in lens epithelium of old rat.



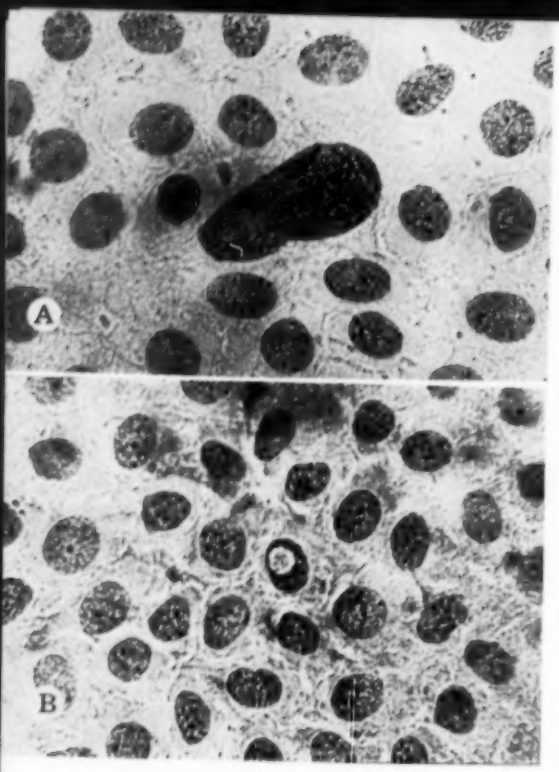
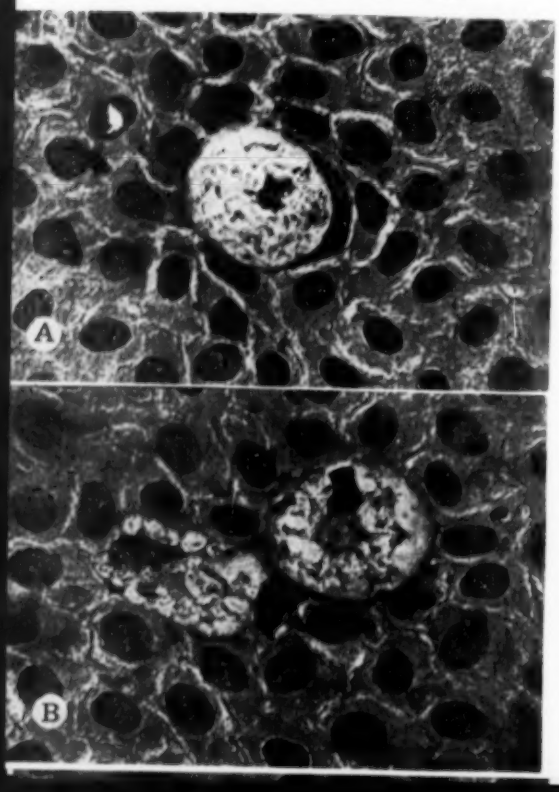


Fig. 19.—*A*, giant nucleus in lens epithelium of old rat. *B*, vacuole in nucleus of cell in lens epithelium of old rat.

Fig. 20.—Excessive vacuolar degeneration of cytoplasm in lens epithelium of old rat.



dose range of 2000 rep 2 to 5 weeks, after exposure. Irradiation with x-rays in the 1000 dose produced beginning opacities in the posterior cortex after five months. The time elapsing between exposure to 2000 r x-rays and development of lens opacities in a group of 52 rabbits amounted in 36 animals to 7-10 weeks and in 16 animals to 4-6 weeks. The latent period, then (prior to initial cataractous changes), was as a rule considerably shorter in eyes bombarded with the heavy particles than in the x-ray series.

COMMENT

In this study the effects of radiations of different linear energy transfers on cell division of the rabbit lens epithelium were evaluated by comparing mitotic cell counts in stained whole mounts from irradiated and control eyes at various time intervals following treatment. Carlson [7] and others emphasized repeatedly the limitations of a procedure of this kind for determining relative radiosensitivities of phases in the mitotic cycle, as well as acceleration or slowing of cell division by irradiation. It was beyond the scope of this report to search for the mentioned aspects of radiobiologic mechanisms, and it was felt that the experimental results were not made less valid by the use of methods which alone are applicable for in vivo studies of the lens epithelium.

Striking responses of cells in the germinative zone were demonstrated by mitotic and degenerate cell counts after exposure of this zone to a circumscribed deuteron beam, whereas the respective counts in the central area were of a low order. Both criteria employed for collating the radiosensitivities of the two parts of the epithelium pointed to a high vulnerability of the cells in the "mitotic area" and to a relative radiotolerance of the cells in the large central part of the epithelium. It is possible that this difference was slightly exaggerated, since the irradiated peripheral zone measured about 36% more than the central one. The results confirmed observations of previous

experiments wherein the entire population of the lens epithelium was irradiated with the 20 mm. x-ray beam, and are in line with abundant reports on radiation effects in "mitotic areas" in which growth rate or proliferative capacity of the tissue seems to determine its radiosensitivity.

Long-term experiments on irradiated animals were few in number but indicated that radiation damage to the equatorial zone of the lens has to be considered a cataractogenic factor. Goldmann and Liechti's [8] experiments with small beams of x-rays, the results reported from this laboratory on irradiation of parts of the limbus with beta particles,[9] and Alter and Leinfelder's [10] and Puntenney and Shoch's [11] data on exposure of portions of the lens to x-rays through various apertures of lead shields denote, on clinical grounds, the high radiosensitivity of the equatorial zone of the lens.

The fact that all dividing and degenerate cells in the entire population of the epithelial layer could be counted and, with regard to cell division, could be related to the normal mitotic index of the epithelium of the non-irradiated eye in the same animal made the lens epithelium particularly suitable for quantitation of the responses; in addition, the radiation factors were well standardized, as described in the section of technique. In spite of the advantages which this material and method offered, the number of mitoses and cells with injury to the nuclei varied widely in individual groups in all three categories. It is known that even uniform groups of cells in a known mitotic stage do not react equally to radiation; consequently, aberrant results are to be expected when tissue with cells in unknown stages of division is irradiated in vivo.

It can be assumed, then, either that the radiosensitivity of cells in corresponding areas in the lens epithelium of animals of the same breed and of similar age varies greatly, or that effects on cell division and on onset and increase of cell damage in reference to zero time frequently follow patterns different from those expressed in averages at the set time intervals. The latter explanation seems to be supported by the less marked variability in the development of lenticular opacities caused by ionizing radiations. Furthermore, differences in the number of degenerate cells in preparations of identically treated eyes may be explained by a varying loss of stainability of the extruded Feulgen-positive material and by interference of the absolute counts at a given time by subsequent waves of such degenerations. To assess on a cellular level the early responses to radiations with different LET's, averages of the variations of the effects in the individual groups were weighted and subjected to statistical analysis: The results indicated an RBE of 4.1 for alpha particles versus x-rays and of 3.9 for deuterons versus x-rays.

Spear and Tansley [12] and Tansley, Gray, and Spear [13] used frog tadpoles and developing retinas of rats for comparing slightly and strongly ionizing radiations. Interference with mitotic activity by x-rays or gamma rays and by neutrons was of similar magnitude, whereas the incidence of neutron-induced cell degeneration exceeded by far that produced by gamma rays. Comparison of some biological effects of alpha radiations with those of gamma rays on the frog tadpole was confined to a preliminary note, but a similar contrast in the effects of the densely and weakly ionizing radiations was stressed. No attempt was made to estimate a value for the RBE of the radiations studied in these experiments. In the present study degree and duration of the delay of mitotic activity, depending on the dose, was also shown to be nearly of the same order for the radiations with dense ionization per micron path and for x-rays. The subsequent increase of mitotic activity in the present study, however, which reached higher levels after bombardment with heavy particles than after x-irradiation, contrasts with the absence of an overshoot phenomenon in the neutron-irradiated developing retina of the rat, as reported by Spear and Tansley. The diversity of the observations on the lens

epithelium and on other biological material in which the number of dividing cells never exceeds the normal after radiation-induced prolonged inhibition of mitosis may be caused by basic biological differences of cell types and tissues in case they were not produced by dissimilarities of the used radiation, doses, and dosimetry.

Spear and Tansley observed in the x-ray and gamma-ray experiments that the latent period which preceded the appearance of degenerate cells increased when the dose was increased. This phenomenon did not take place in their experiments with neutrons, as the degenerate cells became visible at one time, which did not change with the dose. The authors concluded that radiation effects on potentially dividing cells account for the cell degeneration after x-irradiation, but this explanation was not applicable to the extent of cell injury following the exposure to highly ionizing radiations. Here the breakdown of cell nuclei seems to occur independently of preparatory states of cell division. At variance with these results were those of the x-ray experiments on the lens epithelium of rabbits treated with doses ranging from 125 to 2000 r, wherein the latent period between exposure and the onset of cell degeneration did not exhibit dose dependence. On the other hand, there is good agreement between our observations on the lens epithelium and those of the British workers with respect to the breakdown of cell nuclei after treatment with densely ionizing radiations. In the central part of the rabbit lens epithelium the number of degenerate cells surpassed considerably the low mitotic counts in this area when high-energy radiations were administered in doses of 500 rep and upward. Redundancy of damaged nuclei as compared with incidence of mitosis was noticed also in the peripheral zone, suggesting that radiation-induced nuclear fragmentation did not occur only in dividing cells or in cells which attempted division unsuccessfully. Lasnitzki [17] described nuclear fragmentation in chick fibroblasts in the resting phase after irradiation with x-rays in doses above 2500 r. It is doubtful that such effects took place in the lens epithelium with doses up to 2000 r.

In view of the notable variations in response of even homogeneous substrates to ionizing radiations, a great number of eyes were used for the cytological work and its statistical evaluation. Development of radiation cataract in long-term experiments was studied, by necessity only in a short series. These observations provided supporting evidence that radiation-induced damage to the epithelial cells is correlated with the onset and progression of lenticular opacities: The latent period between time of irradiation and onset of initial cataractous changes was shorter, and their progression speedier, in eyes which were bombarded with the high-energy particles than in those treated with the same doses of x-rays.

Size and clear definition of structural details of the cells in the monolayered epithelium make it particularly suitable for mitotic phase counts and for the study of onset and course of cell degeneration. As in other biological material (plants, marine eggs, neuroblasts of grasshopper), effects of ionizing radiations were seen at the first mitosis after treatment (with 1500 r x-rays). Primary or "stickiness" effects were evident 20 minutes after treatment when, in a proportion of cells in anaphase or telophase, sister chromatids or different chromosomes appeared to adhere to each other and formed single or multiple bridges. At the 40- to 50-minute intervals all the remaining cells in division exhibited these signs of sister chromatid fusion.

Clumping of chromatin of the nucleus as an early radiation effect has been described repeatedly in various biological materials. In the cells of the lens epithelium it is not possible to determine whether the change is the result of adherence of different chromosomes to each other. It leads to a variety of degenerate forms and is first seen—soon after treatment—when mitotic activity is at zero. Spear and Glücksmann [14] and Glücksmann and Spear [15] reported exten-

sively on these lesions produced by gamma radiations on the frog tadpole; Glücksmann and Tansley [16] studied such cell degenerations in the rat's developing retina, and Buschke, Friedenwald, and Moses [17] gave an accurate account on the occurrence of nuclear fragmentation in the rat's corneal epithelium after ultraviolet irradiation. Alberti and Politzer # referred to similar x-ray-induced changes in studies of the corneas of urodele larvae. Strangeways and associates * and, later, Lasnitzki † characterized similar forms of nuclear degeneration in irradiated tissue cultures of chick fibroblasts. These are a few examples from the extensive literature on this subject. The morphology of radiation-induced damage to cell nuclei of the lens epithelium deserves special interest, as it adds to the understanding of the pathogenesis of radiation cataract.

In the description of radiation-induced cell degeneration in the lens epithelium, mention was made of the difficulty in evaluating the severity of the nuclear damage in early stages. To distinguish betwen lethal and reversible cell effects, it will be necessary to extend the study to later stages and to other animal species. Whether the nonlethally damaged cells of the germinative zone are those which migrate and give rise to "freak" lens fibers at the time of differentiation is a moot question, but the latent period between radiation insult and cataract formation and microscopic observations in late stages support the hypothesis.

The search for quality effects of the densely and slightly ionizing radiations on cells of the rabbit lens epithelium was unsuccessful. Various rare forms of cell degeneration, such as multipolar karyokinesis or excessive vacuolar degeneration of the cytoplasm with or without nuclear fragmentation, were seen more frequently after bombardment of the eye with high-energy particles, but occurred rarely in the x-ray series. Multipolar cell division was observed in other irradiated tissues, as by Shields Warren [21] in the intestinal tract and by Politzer [23] and Alberti and Politzer ‡ in the cornea of urodeles. Politzer stated that he had encountered such abnormal cell division not more than 10 times in about 10,000 normal urodele corneae. This is in agreement with the negative results of the extensive cytological studies carried out in this laboratory on the lens epithelium of normal young animals of various species. It was interesting, then, to detect tripartite dividing cells in the lens epithelium of rats of very old age. Other age-induced degeneration in this cell layer of rats resembled closely that observed in later stages of radiation-induced damage to the rabbit lens epithelium: poikilocytosis, anisochromasia, giant nuclei and micronuclei, multinuclear cells, and extreme vacuolar degeneration of the cytoplasm. Vacuoles in the nucleus, seen occasionally in the lens epithelium of old rats, were described as radiation damage in the intestinal crypts by Warren, Holt, and Sommers [22] and in the liver by Brues and Reitz.[26] There is, consequently, a striking analogy between radiation- and age-induced cytopathology of the lens epithelium. These lesions precede development of radiation cataract and also may be the initial stage of senile cataract. The conclusion that they present an important pathogenetic factor in various types of lens opacifications appears justified.

References 24 and 25.

* References 19 and 20.

† References 17 and 18.

‡ References 24 and 25.

SUMMARY

Selective exposure of the equatorial and the central area of the lens to the deuteron beam confirmed the impression of high radiosensitivity of cells in the germinative zone—"the mitotic area"—of the epithelium in contrast to relative radioresistance of cells in the large central part.

The effects of densely ionizing radiations (alpha particles and deuterons) and x-rays on cell division and cell degeneration in the

lens epithelium of young rabbits were compared. For the five doses and six time intervals (from 1 to 21 days) the average of mitotic and degenerate counts were weighted, and the specific RBE for these effects $\frac{\text{(rep of alpha particles or deuterons)}}{\text{(rep of x-rays produced by 210 kv.)}}$ was determined. In general, no significant difference in the effects of the two types of radiation on cell division (onset, delay, and overcompensating increase) was noted, but injury produced in the cell nuclei by isodoses of high-energy radiations greatly exceeded that of x-rays.

The RBE of x-rays versus alpha particles for this radiation effect was statistically calculated as 4.1, varying from 2.7 to 6.1 at a confidence range of 99%. The RBE of x-rays versus deuterons was calculated as 3.9 and varied from 2.3 to 6.6 at the same confidence range. The effects of alpha particles and deuterons were of similar magnitude.

Long-term observations of a brief series indicated that, following bombardment of the equatorial zone or of the entire lens with the same dose, in reps, of high-energy particles, the latent period between exposure and initial cataractous changes is much shorter than that following irradiation.

As in other tissues, the earliest radiation-induced morphological changes, observed at the 20-minute interval, consist of chromatin bridges in anaphase and telophase of cells which had entered the mitotic cycle at the time of exposure. Extrusion of chromatin material from the cell nucleus often leads to the death of the cell. On the other hand, cells in which this sign of damage is less extensive may survive. Depletion of the cell population by death of the cells and failure in the differentiation of damaged cells seems to play a part in the eventual development of radiation cataract.

The cell degeneration caused by alpha particles and deuterons differs quantitatively but not qualitatively from that caused by x-radiation. Studies on age-induced cell degeneration in the lens epithelium of rats revealed a striking resemblance between the cytopathology of this cell layer in old age and that of lenses exposed to ionizing radiations.

Dr. G. Failla, Professor of Radiology, Columbia University College of Physicans and Surgeons, gave guidance and constructive criticism in this study

ADDENDUM

Statistical Analysis of Data On Effects of Alpha Particles, Deuterons, and X-Radiation on the Rabbit Lens

JOHN E. SILSON, M.D., M.P.H.

A statistical analysis of both the mitotic and the degenerate cell counts was made to determine confidence ranges for the mean relative radiobiological effectiveness of the three types of radiation on the lens. Statistical methods for pooling the data from different days and different dosages were used in order to obtain the smallest possible standard error.

A. MITOTIC COUNTS

The data on mitotic counts represented in each case the ratio of mitotic cells in the irradiated (right) eye to the nonirradiated (left) eye in each rabbit. Counts were available on a variable number of rabbits at a given number of days following irradiation for each dosage. Visual comparison of the graphs for each time period of dosage plotted against the averaged mitotic ratios showed no appreciable differences except for the alpha-particle versus x-ray curves on the 14th day. Only these data, therefore, were analyzed statistically.

It was noted that the ratios between counts on the irradiated and control eyes rose steadily with increasing dosage. A transformation was therefore sought which would enable us to fit a straight line to these data and, provided the slopes were not too dissimilar, to fit lines of common slope to the data from the two different radiations. Standard statistical bioassay methods [27]

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would then be applicable for the determination of relative radiobiological effect.

The average value at each dosage was used without weighting for the number of animals. The doses administered ranged in arithmetic progression from 125 to 2000 rep; hence it appeared logical to use the logarithm to the base 2 of the dose as the abscissa. Since the effect produced had been measured as a ratio of the mitotic counts in the exposed eyes to the counts in the control eyes, a logarithmic transformation of this ratio also suggested itself. A preliminary plotting of the values on double log paper indicated that a fairly good fit would be obtained by this double transformation, and that the slopes for the two radiations were not too dissimilar.

The ratios at each dosage for each radiation were therefore averaged and the averages converted to logarithms. These logarithms were fitted to lines of best fit against the logarithm of the dose, using the method of least squares. There was no significant difference between the two slopes; therefore the two sets of data were then fitted to lines of common slope. The vertical difference between these lines was significant at the 1% level, using the variation of the points about the lines of common slope to determine the variance. Using the standard error obtained from this variance, 95% and 99% confidence limits were then established for the $\log_2$ of the ratio, and these were, in turn, converted back to ratios. The results are summarized in Table 8.

TABLE 8.—*Ratio of Mitotic Counts in Normal and Exposed Lenses of Rabbits on the Fourteenth Day*

Dose	$\log_2$ Dosage / 500	$\log_{10}$ Ratio: Alpha Radiation	$\log_{10}$ Ratio: X-Radiation	$\log_{10}$ Ratio: Difference	$\log_{10}$ Ratio: Common Slope
125	−2	0.1356	0.0461		
250	−1	0.2348	0.0980		
500	0	0.4646	0.3139		
1000	1	0.5530	0.3832		
2000	2	0.5981	0.4101	.....	.....
Average		0.3952	0.2498	0.1454 *	.....
Slope		0.1223	0.1015	0.0208 †	0.1119

Summary

$\log_2$ of ratio of effects $\frac{\text{(average dose)}}{\text{(common slope)}}$	1.299
S.E. of $\log_2$ ratio ($d = 7$)	0.351
Ratio of effects (antilog)	2.460
95% confidence range	1.386 to 4.386
99% confidence range	1.051 to 5.764

* Statistically significant at 1% level.
† Not statistically significant.

The use of the logarithmic transformation for the degenerate cell counts did not seem advisable, however, because some of the counts were zero. Since the counts were based on area, the use of a square-root transformation suggested itself.

It was noted that for all rabbits killed on the same day the average counts rose with increasing dosage, and that the plot of the square root of this count against the logarithm of the dose was consistently linear for all radiations on all days. For greater accuracy, the mean of the square roots of the counts at each dose of a given radiation

B. DEGENERATE CELL COUNTS

A variable number of lenses were available for each type of radiation, period after exposure, and dosage. It was felt that some method which would combine all these observations would yield a radiobiological effect with the smallest possible standard error. As with the mitotic counts, a $\log_2$

on a given day was used instead of the square root of the mean, in order to reduce the effect of wide variation among the individual readings. No attempt was made to weigh for the number of observations at each point.

Lines were then fitted by the method of least squares to the five doses for each radiation on each day. While there was considerable difference in the slopes for the same radiation on different days, the slopes for different radiations on the same day showed satisfactory parallelism. Paired radiations (alpha particles and x-rays, etc.) were therefore adjusted to lines of common slope for a given day and the average difference in counts calculated from the distances between these lines. The variance of this difference was calculated from the deviations of the average points about the lines of common slope for each day. Since the data were given as differences in counts of degenerate cells at a given dosage, and the results desired were the ratio of dose effects between radiations, the differences in counts for each day were converted to differences of the logarithms of the doses by dividing by the average slope for that day, and the variances appropriately converted.

The differences in effect for a given pair of radiations over the six observation days were then weighted by the reciprocals of their respective variances, summed, and divided by the sum of the reciprocals of the variances to obtain a weighted average over the six days. The reciprocal of the sum of the reciprocals of the variances was used as the variance of the weighted average, and the square root of this, as the standard error, with 42 degrees of freedom (seven for each day). This gave a weighted average and standard error for the logarithms of the dosage ratios, which was readily converted to the relative radiobiological effect. Confidence ranges of 95% and 99% were then determined by subtracting and adding the appropriate factor times the standard error

TABLE 9.—*Relative Radiobiological Effectiveness of Alpha Radiation and X-Radiation on Different Observation Days*

Day	Common Slope	Log$_2$ Ratio	Ratio	S. E. of Log Ratio	Weighting Factor
2	2.240	2.228	4.68 *	0.622	2.58
4	1.465	3.918	15.12 *	0.553	3.27
6	1.500	3.627	12.35 *	1.143	0.77
10	2.760	1.931	3.81 *	0.624	2.57
14	3.900	1.697	3.24 *	0.470	4.53
21	5.105	1.226	2.34 *	0.361	7.07
Weighted average			4.081	0.216	

95% confidence range = 3.614 to 5.525
99% confidence range = 2.719 to 6.126

* Statistically significant at 1% level.

TABLE 10.—*Relative Radiobiological Effectiveness of Deuteron Radiation and X-Radiation on Different Observation Days*

Day	Common Slope	Log$_2$ Ratio	Ratio	S. E. of Log Ratio	Weighting Factor
2	2.330	1.833	3.56 *	0.693	2.14
4	1.945	3.290	9.72 †	1.073	0.87
6	2.510	3.206	9.21 †	1.079	0.86
10	2.225	2.084	4.24 †	0.447	5.00
14	4.000	1.245	2.37	0.672	2.21
21	4.385	1.227	2.51	0.772	1.68
Weighted average			3.874	0.280	

95% confidence range = 2.619 to 5.732
99% confidence range = 2.289 to 6.557

* Statistically significant at 5% level.
† Statistically significant at 1% level.

to the weighted average of the logarithm of the dosage ratios and then converting these values to ratios.

Alpha Radiation Versus X-Radiation

No significant differences were found in the slopes of the two radiations on comparable days, except for the 10-day values, where the difference was significant at the 5% level. The differences between the lines of common slope were highly significant on all six days (well beyond the 1% level). The weighted average for all six days gave a relative radiobiological effect of 4.081 for alpha radiation against x-radiation, which was statistically significant at the 1% level. The data are summarized in Table 9.

Deuterons Versus X-Radiation

No significant differences were found in the slopes of the two radiations on comparable days, except for the 6- and 10-day values, where the differences were significant at the 5% level. The differences between the lines of common slope were highly significant for the first four days, though not as good as for alpha radiation x-rays, but were not statistically significant for 14 and 21 days. The weighted average for all six days gave a relative radiobiological effect of 3.874 for deuterons against x-radiation, which was statistically significant at the 1% level. The data are summarized in Table 10.

Alpha Radiation Versus Deuterons

No significant differences could be found between the two effects on any of the days.

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Tonography

Technical Difficulties and Control Studies

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This paper presents, first, some of the difficulties encountered by us while instituting Grant's method [1] of electrical tonography for clinical use and, second, certain control studies done on normal eyes in an attempt to determine the accuracy of the method as employed by us for routine use. The difficulties described are both the technical ones involved in actually setting up our apparatus and those of technique which led to errors in tracings after our apparatus had been assembled. We are discussing some of these pitfalls with the intent of helping others, beginning in tonography, to avoid them.

Once these difficulties had been surmounted and our method of procedure seemed sufficiently reliable, we studied a series of normal eyes, to obtain data on the reliability of tonography as used by us. Recordings were made on the same eye at different times of the same day, and on different days. Likewise, normal eyes of persons in various age groups were compared to determine the effect of age upon aqueous outflow. The value and the range of variation of the facility of aqueous outflow and the rate of aqueous formation were compared. Studies also were done to compare the accuracy of the above values when calculated from tracings made by a Sanborn recorder with those made from readings taken directly from the electronic tonometer, hoping to determine whether or not a recording apparatus was necessary for reliable tonographic studies. Such a comparison seemed of value because electrical tonography done with a Sanborn recorder involves considerable technical difficulty, which can be avoided by the simpler method of taking readings directly from the tonometer. The data obtained from our various categories of studies done on normal eyes are published to add to those which already have appeared in the literature on tonography because, as with any new method, conclusions on diseased eyes can only be based upon extensive control observations * on normal eyes.

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HISTORICAL REVIEW

Kronfeld [8] has recently reviewed the development of tonography. He stated that tonography is a new term for a procedure which consists essentially of the observation of the drop in ocular tension that occurs during prolonged or sustained application of a tonometer of the Schiötz type to the cornea. The first detailed study of the effect of prolonged application of a tonometer to the eye was reported by Wegner [11] in 1925. Other articles have appeared in the European literature, but the first to be published in this country was by Böck, Kronfeld, and Stough,[12] in 1934, who reported a statistical drop of the ocular tension that occurred in 100 nonglaucomatous eyes under the influence of a tonometer load of 15 gm. applied for a two-minute period. Moses and Bruno [13] reported a method of measuring the rate of outflow of aqueous fluid from the eye, using either a

* References 1 to 10

certified Schiøtz or a Mueller electronic tonometer and applying the values obtained to Friedenwald's data. A permanent record was made by photographing the dial of the Mueller electronic tonometer while the instrument was applied to the eye. Grant,[1] in work done simultaneously (1950), developed his method of tonography for measuring the facility and the net rate of aqueous outflow. He obtained a continuous recording of the ocular tension by applying a Mueller electronic tonometer, attached to a Sanborn strip recorder, for five or six minutes. Using data of his own and Friedenwald's, he determined the steady-state pressure before application of the tonometer, the pressure during the measurement procedure, and the volume of fluid expressed from the eye. From these figures, he was able to calculate the facility of aqueous outflow in terms of cubic millimeters per minute per millimeter Hg pressure. He has since carried out extensive studies in normal and glaucomatous eyes, as well as upon enucleated eyes.† Increasing numbers of reports, utilizing Grant's method, are now appearing in the literature. The data reported in this paper are obtained, using the apparatus and formulas introduced by Grant. The formula for the coefficient of facility of aqueous outflow is $C = \frac{\Delta V}{T\ (\text{Average } Pt\text{-}Pv)}$ where C is the coefficient, expressed as cubic millimeters per minute per millimeter Hg pressure, ΔV is the change in ocular volume for the initial and final tonometric readings, T is time, in minutes, Pt is intraocular pressure during tonography, and Po is intraocular pressure immediately before tonography. The formula for the net rate of flow of aqueous is $K = C\ (Po\text{-}Pv)$, where K is the net rate of flow, expressed as cubic millimeters per minute, C is the coefficient of facility of aqueous outflow, Po is the intraocular pressure immediately before tonography, and Pv is the episcleral venous pressure. In 1951, Goldman [6] determined the value for Pv (episcleral venous pressure) to be approximately 8 to 10 mm. Hg, and in 1953 Becker and Friedenwald [14] presented data indicating that Pv was essentially unchanged during tonography. In a recent report, Grant [4] used a value of 8 mm. Hg, instead of the previous 4 mm. Hg, in calculating K. We also used a value of 8 mm. Hg for Pv in calculating K. In the remainder of this paper the coefficient of facility of aqueous outflow will be referred to as C, and the net rate of flow as K, to avoid needless repetition.

† References 1 to 5.

PRECAUTIONS—POSSIBLE SOURCES OF ERROR IN TECHNIQUE

Strict attention to details is essential for obtaining reliable tonographic tracings. The following precautions must be observed.

A. Preparation of Patient.—Tonography should be done in a quiet room, where the patient's attention will not be diverted. He should be placed on an examining table, with his head resting comfortably on a pillow, to help prevent restlessness during the study. A tight collar or necktie should be loosened to insure free venous flow from the head and neck. Any interference with venous circulation results in a distorted tracing and inaccurate values (Fig. 1). The patient should have the nature of the procedure explained to him and the length of time which it takes so that he will not become fearful during the test.

B. Anesthesia.—Adequate anesthesia can be obtained by the instillation of a local anesthetic agent at three-minute intervals. We employed either Ophthaine (2-diethylaminoethyl-3-amino-4-propoxybenzoate HCl) 0.5%, or tetracaine hydrochloride, 1%.

C. Fixation.—The eye must be quiet during application of the tonometer. Any movement results in an irregular tracing (Fig. 2). The patient is asked to look at a fixation target suspended directly above the eye on an adjustable metal stand. A striped ball, about 10 mm. in diameter, helps to eliminate sensory adaptation and permits better fixation than a target of solid color.

D. Retraction of Lids.—The lids must be retracted from the eyeball, so that the effect of lid movements is not transmitted to the eye and hence to the tracing. Any pressure exerted through the lids is immediately reflected upon the curve (Fig. 3). Grant retracts the lids with his fingers, but we prefer to use nonmagnetic Castroviejo lid clamps of the type described by him for use in ocular surgery.

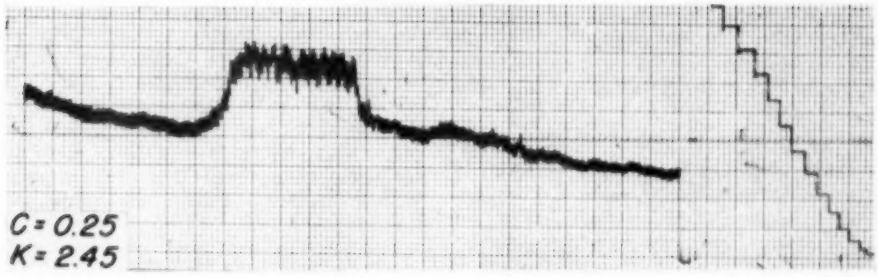

Fig. 1.—Tracing showing irregularity due to interference with venous return from the head and neck (blood pressure cuff around the neck).

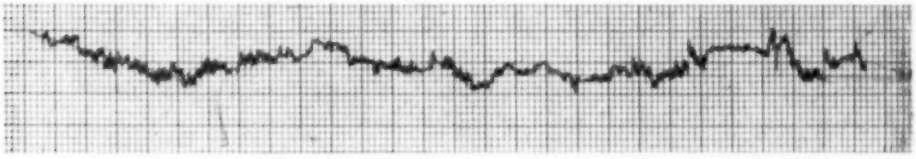

Fig. 2.—Tracing showing irregularity due to movement of the eye as a result of poor fixation.

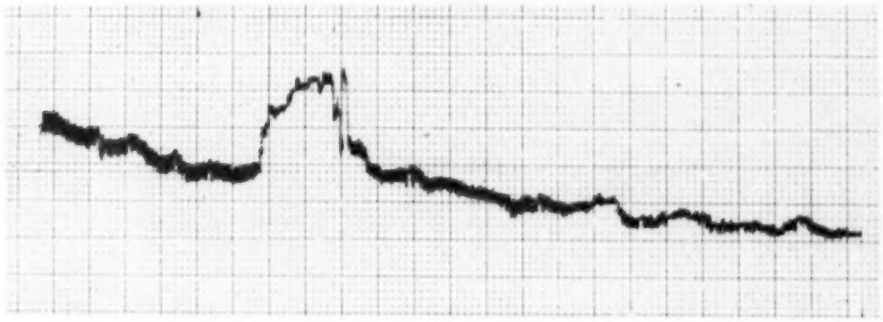

Fig. 3.—Tracing showing irregularity due to patient's squeezing eyelids and straining.

E. Application of the Tonometer.—The cornea should be moistened by several drops of local anesthetic or saline solution just prior to application of the tonometer to help prevent abrasion of the corneal epithelium by the dry foot plate of the tonometer. If the surface of the cornea is not wet, the steel surface of the foot plate, which must be dry when applied, tends to adhere to and damage the epithelium. The tonometer should be poised just above the eye for approximately 20 seconds, and then very slowly lowered onto the cornea, in such a manner that the patient is almost unaware of the moment of contact. This helps to alleviate apprehension on the part of the patient and insures better fixation. Intervals of one minute should be announced during the tracing to encourage the patient as to the lapse of time. The tonometer should be applied with one's hand comfortably placed on the patient's forehead or cheek, so that the tonometer can be held steadily for four minutes. Likewise, one should be comfortably seated.

DIFFICULTIES WITH TONOGRAPHIC EQUIPMENT

Our equipment consisted of the Mueller electronic tonometer and the Sanborn recorder, as recommended by Grant. A full-wave selenium instrument rectifier and a 5-microfarad electrolytic condenser were added to the tonometer, which must be adequately grounded. The Sanborn M-126 direct-current amplifier and the M-127 recorder, which had been modified by the Sanborn Company to meet the specification of the M-124 amplifier and the M-123 recorder, were used.

Several difficulties arose in using the Sanborn recording system in combination with the Mueller electronic tonometer. Some of these, due largely to our inexperience, were overcome only after considerable trouble.

A. Electric Output of Tonometer.—The problem which was most difficult to surmount was caused by our tonometer, which had a voltage output that was too high to permit recording with the Sanborn apparatus. A special resistor jack was introduced between the output of our tonometer and the input of the recording system, to bring the voltage output down to the range of 250 mv., with a scale reading of 7.0 on the tonometer, as required of the apparatus. Figure 4 shows a tracing with and a tracing without the jack condenser in the circuit. The tracing with the jack condenser shows a uniform curve, from which accurate readings can be taken. We later found that the voltage output of individual electronic tonometers varies greatly. In the other instrument which we used, the voltage output was too low for use with the Sanborn recorder system. This was overcome by changing the resistance in the output circuit of the tonometer to increase the voltage output to the range required by the recorder apparatus. At present, it is impossible to buy an electronic tonometer, connect it with a Sanborn recorder, and expect to obtain good tracings. This might serve as a further argument for standardization of these tonometers and the parts of which they are comprised. Variations in the voltage supply of the current to the electronic tonometer may cause variations in the scale readings, but in our laboratory this was not a problem, since the current was stable. Where this is a problem, a constant-voltage transformer of the type recommended by Askovitz [15] is effective. We repeated the experiment of Askovitz to determine the variation in scale readings in our two electronic tonometers with changes in the voltage supply, and our results are shown in Table 1. With the plunger of the tonometer set to give a tonometer reading of 3.0 after calibration, a drop in voltage from 115 to 95 volts produced a change in the scale reading of 0.4 division in Tonometer 1 and 0.1 division in Tonometer 2. When the constant-voltage transformer was used, there was no change in the scale readings with variation in the voltage supply.

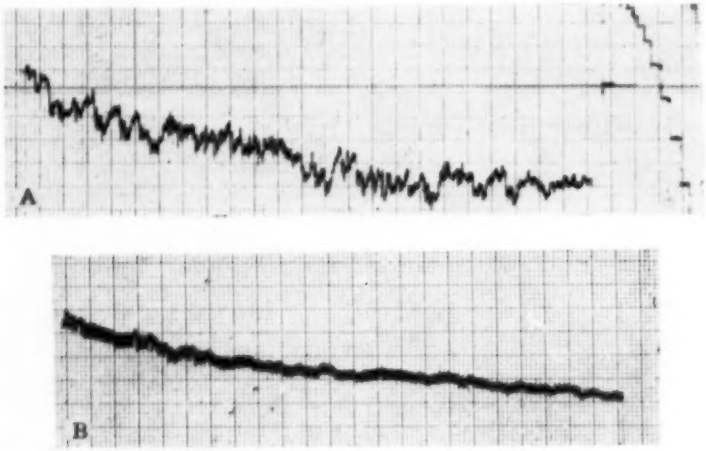

Fig. 4.—*A*, tracing without jack condenser in the circuit, showing grossly irregular fluctuations. *B*, tracing with jack condenser in the circuit, showing uniform type of curve.

CARE OF TONOMETER

Strict care of the tonometer is necessary. The plunger and barrel must be thoroughly cleaned before each tonographic study, because the accumulation of tears and secretions interferes with free movement of the plunger. Originally, we cleaned our instrument with distilled water and dried it with ether. However, over a period of several weeks, the ether dissolved the insulation of the wiring and ruined the tonometer head. At present, we are cleaning the barrel of the tonometer with small squares of lens cloth moistened in saline and held on a wood applicator stick. The plunger is wiped off first with alcohol to remove any oily film which may have accumulated, then with saline, and finally is dried thoroughly with a cotton lens cloth. A lens cloth avoids the deposition of lint and cotton fibers in the barrel of the tonometer.

TABLE 1.—*Variation in the Electronic Tonometer Scale Readings with Changes in the Voltage Supply*

Voltage Input	Electronic Tonometer No. 1 Scale Reading		Electronic Tonometer No. 2 Scale Reading	
	Without Constant Voltage Transformer	With Constant Voltage Transformer	Without Constant Voltage Transformer	With Constant Voltage Transformer
95	2.6	3.0	2.9	3.0
100	2.7	3.0	2.9	3.0
105	2.8	3.0	2.9	3.0
107.5	2.9	3.0	3.0	3.0
110	3.0	3.0	3.0	3.0
112.5	3.0	3.0	3.0	3.0
115	3.0	3.0	3.0	3.0
117.5	3.1	3.0	3.0	3.0
120	3.2	3.0	3.1	3.0
125	3.2	3.0	3.2	3.0

CONTROL STUDIES ON NORMAL EYES

Control studies have been reported by Grant,‡ deRoetth and Knighton,[7] and Kronfeld,[8] but we wished to know how our results compared with their studies and how accurately we could reproduce our own tracings on the same eye. It also seemed of considerable importance to add to the data in the literature on tracings in a large group of normal eyes. It is obvious that the same variability found in normal eyes would have to be assumed to exist in the study of glaucomatous eyes.

A. Studies to Determine Variation in Facility of Outflow in Normal Eyes Determined by Tonography.—1. Variability in Tracings Repeated on Same Eye at Six-Hour Intervals on Same Day: We first attempted to determine the consistency of tonographic measurements performed on the same eye at 6-hour intervals over a 30-hour period. Observations were made on 14 normal eyes (Table 2). The mean value for *C* and the plus or minus percentage of variation from this value is shown for each patient. A range of variation of ± 8% to ± 30% was observed. An average variation of ±0.045 cu. mm/min/mm. pressure, or ± 19% from the mean *C*, was observed on repeated tonographic measurements in this group. No consistent diurnal pattern was noted which could be considered characteristic for any particular time of the day.

2. Variability of Tonographic Values in Daily Tracings Repeated on Same Eye on Five Successive Days at 7 a. m., 1 p. m., or 6 p. m.: Because of the corneal irritation which resulted from a single tonographic study, we shortly stopped making more than one tracing on a single day and, instead, did tracings at the same time of the day, but on different days, on the same eye. The variability in *C* calculated from tracings performed in this manner on 12 normal eyes at 7 a. m., 1 p. m., or 6 p. m. over a period of five different days is shown in Table 3. Values of 16% to 35% from the mean were observed in this group. An average variation of ±0.053 cu. mm/min/mm. pressure, or ±25% from the mean *C*, was noted. These values, as in those previously found over the same 30-hour period at 6-hour intervals, showed no characteristic diurnal variation. Our figures compared favorably with those of Kronfeld,[8] who reported a coefficient of outflow with ±25% of the arithmetic mean in normal eyes of patients tested at different times of the day, and with those of Moses and Bruno,[13] who reported a range of ±30%, as well as of Grant,§ who found a slightly narrower range in the same normal eye.

3. Variability in Tracings Repeated on Normal Eyes at Same Time on Successive Days: The results of 78 tracings performed on 39 different normal eyes at the same time on successive days are shown on Table 4. In 10 tracings on five eyes we were able to obtain exactly the same *C* value on different days, and in 4 tracings on two eyes we found a variation as great as 0.10 cu. mm/min/mm. pressure. Figure 5 shows graphically the range of variation in this study. The average *C* value in the 78 tracings was 0.218 cu. mm/min/mm. pressure. The average variation in *C* on repeat tracings at the same time of the day was ±0.036 cu. mm/min/mm.

‡ References 1 to 5.

§ References 1 and 2.

TABLE 2.—*Lack of Consistent Diurnal Variation in Tonographic Values Over a Thirty-Hour Period*

Name	Eye	7 A.M.			1 P.M.			7 P.M.			1 A.M.			7 A.M.			1 P.M.			Mean C Value	Variation Observed
		T	C	K	T	C	K	T	C	K	T	C	K	T	C	K	T	C	K		
E. K.	O D	14	0.23	1.61	14	0.24	1.12	12	0.16	0.58	..	....	....	13	0.26	1.14	14	0.27	1.38	0.23	± 30%
	O S	13	0.20	0.80	11	0.15	0.42	14	0.20	1.00	..	....	....	12	0.20	0.64	10	0.17	0.29	0.18	± 16%
W. P.	O D	17	0.20	1.36	14	0.30	1.32	11	0.25	0.69	..	....	....	14	0.27	1.19	14	0.31	1.64	0.27	± 26%
	O S	14	0.23	1.10	14	0.28	1.23	10	0.31	0.62	..	....	....	12	0.29	0.90	13	0.31	1.24	0.28	± 11%
C. G.	O D	13	0.21	0.84	16	0.25	1.70	14	0.24	1.37	11	0.17	0.43	..	....	....	10	0.20	0.34	0.21	± 19%
	O S	14	0.25	1.22	13	0.28	1.01	14	0.27	1.32	14	0.29	1.42	..	....	....	12	0.25	0.80	0.27	± 8%
P. T.	O D	18	0.27	2.16	15	0.29	1.54	13	0.25	1.00	8	0.17	0.17	..	....	....	..	....	....	0.24	± 28%
	O S	15	0.24	1.27	12	0.23	0.74	11	0.17	0.41	6	0.17	....	..	....	....	..	....	....	0.20	± 20%
G. R.	O D	20	0.26	2.36	20	0.32	2.84	19	0.30	2.58	17	0.31	2.29	..	....	....	13	0.25	1.00	0.29	± 14%
	O S	15	0.25	1.32	14	0.32	1.42	14	0.34	1.67	13	0.34	1.36	..	....	....	14	0.28	1.37	0.31	± 19%
L. B.	O D	20	0.19	1.75	18	0.20	1.33	16	0.18	1.04	17	0.23	1.36	..	....	....	14	0.22	1.16	0.20	± 10%
	O S	17	0.18	1.22	17	0.20	1.30	14	0.20	1.06	16	0.25	1.58	..	....	....	14	0.22	1.16	0.21	± 19%
J. S.	O D	14	0.23	1.22	12	0.20	0.64	13	0.18	0.72	14	0.27	1.32	..	....	....	..	....	....	0.22	± 23%
	O S	12	0.27	1.19	12	0.18	0.58	14	0.25	1.22	14	0.24	0.86	..	....	....	..	....	....	0.24	± 25%

Average variation from mean C value = ± 19%.
Average variation from mean C value = ± 0.045 cu. mm. /min./mm. pressure.

TABLE 3.—*Lack of Consistent Diurnal Variations in Tonographic Values on Normal Eyes at Different Times on Various Days*

Name	Eye	7 A.M.			1 P.M.			6 P.M.			7 A.M.			1 P.M.			Mean C Value	Variations Observed
		T	C	K	T	C	K	T	C	K	T	C	K	T	C	K		
J. H.	O S	22	0.30	3.18	20	0.21	1.66	22	0.41	4.92	23	0.32	3.62	20	0.30	2.76	0.31	± 35%
R. P.	O S	19	0.28	2.41	16	0.28	1.76	17	0.22	1.59	17	0.20	1.26	17	0.25	1.70	0.23	± 22%
R. P.	O S	17	0.17	1.26	20	0.15	1.38	18	0.13	1.20	19	0.13	1.12	19	0.18	1.55	0.15	± 20%
J. K.	O S	21	0.21	2.37	17	0.15	1.11	19	0.20	1.60	19	0.18	1.44	19	0.17	1.26	0.18	± 17%
R. S.	O S	17	0.16	1.18	19	0.25	2.15	19	0.12	1.44	17	0.20	1.36	17	0.20	1.48	0.20	± 25%
A. S.	O S	19	0.22	1.76	19	0.32	2.56	19	0.25	2.00	20	0.23	2.12	19	0.25	2.15	0.25	± 28%
M. A.	O S	14	0.20	0.98	12	0.24	0.86	14	0.27	1.19	12	0.22	0.79	14	0.27	1.32	0.24	± 16%
R. E.	O S	17	0.16	1.09	19	0.25	2.00	18	0.22	0.88	14	0.13	0.57	17	0.24	2.10	0.20	± 35%
W. F.	O S	10	0.13	0.72	13	0.22	0.88	11	0.20	0.56	11	0.13	0.31	12	0.16	0.51	0.17	± 29%
E. H.	O S	19	0.25	2.00	17	0.33	2.24	17	0.20	1.36	19	0.30	2.58	19	0.25	2.00	0.27	± 26%
E. R.	O D	15	0.19	1.01	19	0.23	1.84	16	0.25	1.58	17	0.18	2.07	17	0.20	1.48	0.21	± 19%
N. W.	O S	17	0.14	0.95	17	0.15	1.11	14	0.16	0.78	16	0.14	0.88	19	0.20	1.76	0.16	± 25%

Average variation from mean C value = ± 25%.
Average variation from mean C value = ± 0.053 cu. mm. /min./mm. pressure.

TABLE 4.—*C, Coefficient of Aqueous Outflow, and K, Net Rate of Flow, in Normal Eyes at the Same Time on Successive Days*

	1st Day		2d Day			1st Day		2d Day	
Eye	C	K	C	K	Eye	C	K	C	K
1	0.28	2.41	0.20	1.26	21	0.20	1.26	0.30	1.14
2	0.16	1.00	0.13	0.57	22	0.23	1.10	0.29	0.86
3	0.22	1.76	0.23	2.12	23	0.30	2.56	0.30	1.84
4	0.16	1.18	0.20	1.36	24	0.19	2.01	0.18	1.55
5	0.19	1.01	0.18	2.07	25	0.28	1.76	0.23	1.70
6	0.25	1.58	0.30	1.47	26	0.32	2.56	0.35	2.15
7	0.14	0.56	0.14	0.88	27	0.25	2.15	0.20	1.48
8	0.32	3.02	0.30	3.18	28	0.23	1.84	0.20	1.03
9	0.17	1.26	0.13	1.12	29	0.16	1.09	0.18	1.33
10	0.25	2.00	0.30	2.58	30	0.23	1.22	0.20	0.98
11	0.20	0.98	0.22	0.79	31	0.18	1.33	0.23	1.22
12	0.21	2.37	0.18	2.16	32	0.21	1.33	0.23	1.27
13	0.13	0.64	0.13	3.10	33	0.15	1.11	0.20	1.60
14	0.22	1.28	0.23	1.98	34	0.21	1.50	0.30	2.70
15	0.17	0.99	0.23	2.16	35	0.15	1.38	0.18	1.55
16	0.30	2.04	0.30	1.74	36	0.33	2.21	0.25	2.40
17	0.30	1.90	0.30	2.40	37	0.24	0.80	0.27	1.40
18	0.23	1.61	0.26	1.14	38	0.15	1.11	0.17	1.26
19	0.20	0.80	0.30	1.44	39	0.22	0.88	0.16	0.51
20	0.11	1.17	0.17	1.92					

Average C, coefficient of aqueous outflow = 0.218 cu. mm. min./mm. pressure.
Average variation in C = ± 0.036 cu. mm./min./mm. pressure = ± 16.5%.

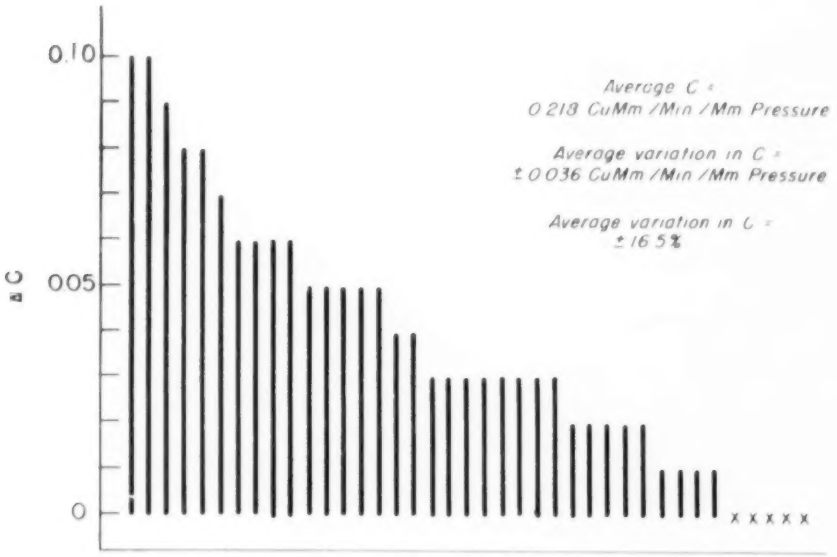

Fig. 5.—Graph showing the variations in C, coefficient of aqueous outflow, in normal eyes at the same time on successive days.

pressure, or ±16.5%, which compared with a variation of ±10% found in six tonograms on a single eye by Kronfeld.[8] The variation in *C* values was less when the tonographic procedure was performed at the same time of the day than when it was done at different times on successive days. This confirms the belief that tonography in repeat studies should be performed at the same time of day, preferably early in the morning, when physical and emotional activities are at a minimum.

over-all average *C* of 0.224 cu. mm/min/mm. pressure and an average net rate of flow of 1.45 cu. mm/min. were found for the 240 tracings on 140 normal eyes. A range of 0.11 to 0.42 cu. mm/min/mm. pressure was found for the coefficient of outflow and 0.17 to 4.92 cu. mm/min. for the net rate of flow. These findings are compared with those of other authors in Table 5 and found to be very similar, with the exception of Goldman,[5] whose values were obtained by different tech-

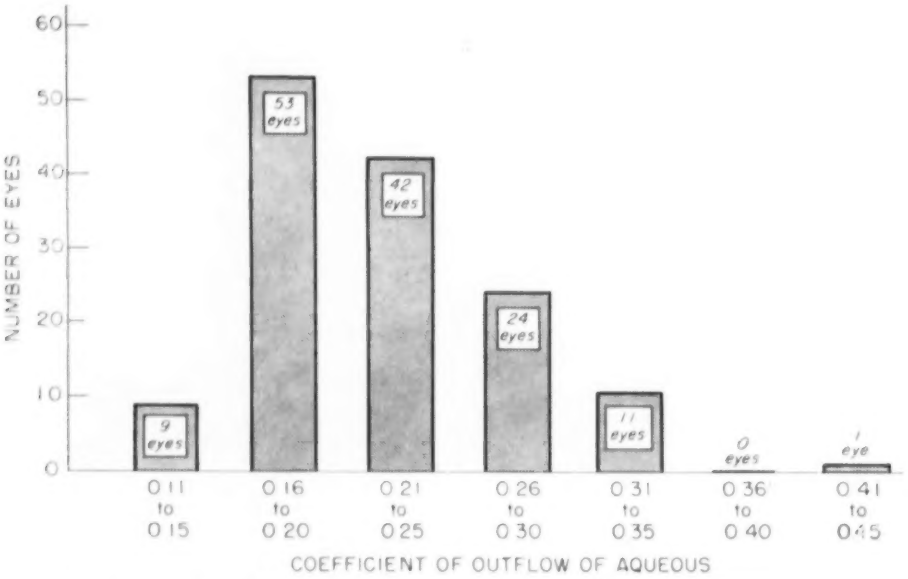

Fig. 6.—Graph showing distribution of coefficient of outflow of aqueous in 140 normal eyes.

4. Values of Coefficient of Aqueous Outflow in Normal Eyes Determined by Single Tracings: The distribution of the values for the coefficient of outflow is of vital interest in helping to determine normal levels for aqueous outflow. Figure 6 shows a distribution of the coefficient of aqueous outflow determined on 140 normal eyes between the hours of 8:30 and 10:30 a. m. An average coefficient of outflow of 0.220 cu. mm/min/mm. pressure was found in this series, with a range of 0.110 to 0.420 cu. mm/min/mm. pressure. In an additional 100 tracings performed on this group of 140 normal eyes, an

niques. We found that 31% of the *C* values fell between the range of 0.100 and 0.199 cu. mm/min/mm. pressure, as compared with 35% in this range in Kronfeld's studies. For any one tracing, a variation of ±16.5% to ±25% must be applied. If this is done for any one of the 31% of normal values for *C* falling in the range of 0.11 to 0.199 cu. mm/min/mm. pressure, an abnormal or borderline result might be obtained. The *C* value was 0.16 cu. mm/min/mm. pressure, or more, in 94% of eyes. It seems justifiable, therefore, to regard any value below 0.16 as suspicious.

TABLE 5.—*Summary of Coefficient of Aqueous Outflow and Net Rate of Flow in Normal Eyes as Reported by Various Authors*

Author	Year	No. of Eyes	Range of Coefficient of Aqueous Outflow, C, Cu. Mm./Min./Mm. Hg Pressure	Average Coefficient of Aqueous Outflow, C, Cu. Mm./Min./Mm. Hg Pressure	Range of Net Rate of Flow, K, Cu. Mm./Min.	Average Net Rate of Flow, K, Cu. Mm./Min.
Grant	1950	34	0.015-0.34	0.243	2.8-5.4	3.66
Grant	1951	118	0.11-0.44	0.22	1.1-5.13	2.40
Goldmann	1951	..	.....	0.33	.....	2.2
deRoetth and Knighton	1952	140 (160 tracings)	0.10-0.52	0.244	1.0-9.3	4.0
Kronfeld	1952	40	0.14-0.44	0.24	.....	....
Mansheim	1953	40	0.15-0.32	0.23	1.05-4.80	3.03
Stepanik	1954	30	0.10-0.36	0.22	.....	....
Grant	1954	171	.....	0.230	.....	1.29*
Spencer, Helmick, Scheie	1955	140 (240 tracings)	0.11-0.42	0.224	0.17-4.92	1.4-7

* With episcleral venous pressure (*Pv*) 8 mm. Hg.

B. Comparison of Tonographic Values Calculated from a Sanborn Tracing with Those Made from Scale Readings Taken Directly from an Electronic Tonometer.—Initially, we calculated the *C* value for each tracing by determining the average *Pt* from the intermediate *Pt* taken at 30-second intervals from the tracing and utilizing Grant's formulas and Friedenwald's tables. On tracings with a smooth curve and uniform drop the *C* value could be calculated just as accurately from Grant's nomograms using the initial and final scale readings. The results obtained by these two methods were then compared with *C* values computed by taking readings directly from the Mueller tonometer scale at half-minute intervals. Table 6 shows that once accurate technique is acquired there is very little difference in the *C* or *K* values obtained by using these three methods. Table 6 shows the *C* value obtained in 30 normal eyes using these three methods. The average *C* obtained by utilizing the initial and final scale readings from the Sanborn recorder tracing was 0.187 cu. mm/min/mm. pressure; the average *C* obtained from readings at half-minute intervals from the Sanborn recorder was 0.197 cu. mm/min/mm. pressure, and the average *C* obtained from readings taken at half-minute intervals directly from the electronic tonometer was 0.201 cu. mm/min/mm. pressure. Occasionally, a tonographic tracing is so irregular that the Sanborn recorder tracing offers distinct advantages in making the calculations. However, the use of the electronic tonometer without the recording apparatus would be less expensive, easier, and in most instances, just as accurate.

TABLE 6.—*C, Coefficient of Aqueous Outflow, Derived by Various Methods of Obtaining Scale Readings*

Pt.	Eye	C Readings Sanborn Recorder Initial and Final	C Readings ½ Min. Interval Sanborn Recorder	C Readings ½ Min. Interval Direct from Electronic Tonometer
J. H.	OS	0.32	0.35	0.33
M. J.	OD	0.16	0.16	0.20
M. J.	OS	0.17	0.17	0.14
R. P.	OS	0.13	0.13	0.13
L. H.	OS	0.15	0.16	0.18
A. A.	OS	0.15	0.14	0.13
A. H.	OD	0.23	0.29	0.27
A. H.	OS	0.20	0.23	0.22
E. R.	OD	0.20	0.22	0.23
E. R.	OS	0.25	0.29	0.26
S. W.	OS	0.20	0.22	0.22
E. M.	OD	0.15	0.14	0.14
E. M.	OS	0.20	0.23	0.26
R. S.	OS	0.11	0.11	0.13
A. D.	OS	0.40	0.40	0.38
M. R.	OS	0.22	0.24	0.28
D. G.	OD	0.17	0.16	0.18
R. M.	OS	0.13	0.13	0.15
A. S.	OD	0.28	0.27	0.31
A. S.	OS	0.16	0.17	0.19
F. S.	OD	0.20	0.22	0.24
F. S.	OS	0.16	0.15	0.17
R. C.	OS	0.15	0.15	0.17
A. B.	OD	0.20	0.21	0.17
A. B.	OS	0.13	0.13	0.13
T. P.	OS	0.12	0.14	0.13
I. R.	OD	0.17	0.17	0.17
I. R.	OS	0.19	0.19	0.17
R. C.	OD	0.14	0.14	0.14
F. G.	OS	0.18	0.19	0.22
Average		0.187	0.197	0.201

TABLE 7.—*No Significant Change in C, Coefficient of Aqueous Outflow, or K, Net Rate of Flow in Various Age Groups*

11-20 Yr.	OD		OS	
Patient	C	K	C	K
B. C.	....	....	0.23	2.27
B. W.	....	....	0.21	2.23
J. T.	0.18	0.65	0.20	0.72
R. K.	0.20	1.36	0.13	0.75
D. V.	0.27	2.00	....	....
M. F.	....	....	0.25	1.58
G. L.	0.23	2.25	0.20	1.84
A. K.	....	....	0.35	3.22
S. B.	....	....	0.30	2.20
J. M.	....	....	0.16	1.28
A. W.	....	....	0.18	0.88
E. U.	0.16	0.78	0.20	0.88
R. B.	0.13	0.82	0.16	1.09
D. G.	0.28	1.90	....	....
D. G.	0.17	1.16	....	....
Average	C = 0.21		K = 1.48	

21-30 Yr.	OD		OS	
Patient	C	K	C	K
R. P.	....	....	0.17	1.26
J. H.	0.30	3.38	....	....
N. W.	....	....	0.14	0.85
B. P.	0.29	1.68	0.20	0.64
M. E.	0.42	2.23	....	....
T. C.	0.25	1.58	....	....
R. S.	....	....	0.16	1.18
A. S.	....	....	0.22	1.76
R. E.	....	....	0.16	1.09
E. H.	0.30	3.49	0.24	2.71
J. G.	0.22	1.28	0.17	0.90
R. P.	0.23	2.12	0.28	2.41
A. A.	0.30	0.51	....	....
E. S.	0.25	2.45	0.18	1.13
G. N.	0.25	1.85	....	....
Average	C = 0.24		K = 1.88	

31-40 Yr.	OD		OS	
Patient	C	K	C	K
E. B.	0.25	1.28	....	....
E. R.	0.19	1.01	....	....
E. H.	....	....	0.25	2.00
E. F.	0.31	3.97	....	....
M. J.	0.30	2.40	....	....
C. G.	0.21	0.84	....	....
G. R.	0.26	2.39	0.25	1.32
J. G.	0.16	1.92	0.15	0.79
M. A.	....	....	0.20	0.86
J. K.	....	....	0.21	2.37
W. F.	....	....	0.13	0.22
I. R.	0.17	1.56	0.19	1.29
M. T.	0.23	1.33	0.33	3.23
P. G.	0.23	0.92	0.22	0.79
J. H.	0.17	1.46	....	....
Average	C = 0.22		K = 1.59	

41-50 Yr.	OD		OS	
Patient	C	K	C	K
S. D.	0.26	1.64	0.20	0.98
C. L.	0.25	1.00	....	....
W. L.	0.22	1.76	....	....
P. T.	0.27	2.16	0.24	1.27
T. D.	0.11	1.17	0.15	1.28
W. P.	0.20	1.36	0.23	1.10
M. H.	0.32	2.56	0.33	2.84
E. J.	0.28	3.01	0.27	1.70
A. B.	0.32	2.02	....	....
H. S.	....	....	0.16	0.76
V. S.	0.30	1.89	0.25	1.32
G. L.	0.26	2.55	0.31	1.24
	....	....	....	....
	....	....	....	....
	....	....	....	....
Average	C = 0.25		K = 1.60	

51-60 Yr.	OD		OS	
Patient	C	K	C	K
M. W.	0.33	1.32	0.30	1.08
F. M.	0.17	0.75	....	....
E. W.	0.30	2.04	0.30	1.90
J. S.	0.31	1.52	0.34	1.67
E. K.	....	....	0.20	0.80
B. M.	....	....	0.18	1.04
J. S.	0.20	3.26	0.17	1.92
M. K.	0.18	1.22	0.14	0.56
M. C.	....	....	0.16	1.09
L. H.	0.21	2.37	0.20	1.48
M. S.	0.30	1.47	....	....
C. D.	0.18	1.04	0.16	0.78
E. W.	....	....	0.25	1.00
Average	C = 0.21		K = 1.41	

61-70 Yr.	OD		OS	
Patient	C	K	C	K
P. B.	0.22	0.80	....	....
M. J.	....	....	0.23	1.98
R. R.	0.25	1.22	....	....
H. S.	0.18	1.55	0.18	1.13
E. A.	0.20	1.69	0.22	1.63
A. M.	0.22	1.63	0.24	1.27
L. B.	0.19	1.75	0.18	1.22
M. L.	0.25	1.00	0.25	0.60
P. R.	0.27	1.08	....	....
M. J.	0.25	1.45	0.20	0.80
E. S.	....	....	0.20	3.02
R. R.	0.27	1.19	0.25	1.00
T. B.	0.22	0.44	....	....
Average	C = 0.21		K = 1.32	

71-80 Yr.	OD		OS	
Patient	C	K	C	K
R. K.	0.23	1.22	....	....
A. C.	0.16	0.93	....	....
J. S.	0.20	2.32	....	....
L. K.	0.28	2.78	0.20	0.81
A. K.	0.25	1.23	0.25	1.23
R. H.	0.24	2.21	0.18	0.88
H. S.	0.18	1.22	0.15	0.73
J. L.	0.17	0.90	0.20	0.64
A. C.	0.20	0.48	0.18	1.33
C. M.	0.18	0.95	0.22	0.79
M. O.	0.28	2.41	0.20	1.36
J. L.	0.18	2.72	....	....
	....	....	....	....
Average	C = 0.21		K = 1.34	

Average C, coefficient of aqueous outflow = 0.22 cu. mm. min. mm. pressure.
Average K, net rate of flow = 1.52 cu. mm. min.
Average C in patients greater than 50 years of age = 0.21.
Average C in patients less than 50 years of age = 0.23.

C. Influence of Age of Patient on Facility of Aqueous Outflow.—Bock, Kronfeld, and Stough [12] raised the question of the influence of the age of the patient on the drop of intraocular tension under the influence of protracted tonometry. They concluded that there seemed to be no tendency of the eyes of older persons to show small drops in tension more frequently than those of persons under 50. Grant [2] found that age was not a factor in determining either the facility of the aqueous outflow or the rate of formation of aqueous. results of these studies. Figure 7 shows graphically that there is a tendency for the C and K values to be decreased in patients past the age of 50; however, this slight decrease does not appear to be significant. In this study the patients past the age of 50 years showed an average C value of 0.21 cu. mm/min/mm. pressure and an average K value of 1.36 cu. mm/min., as compared with an average C value of 0.23 cu. mm/min/mm. pressure and an average K value of 1.64 cu. mm/min. in the patients less than 50

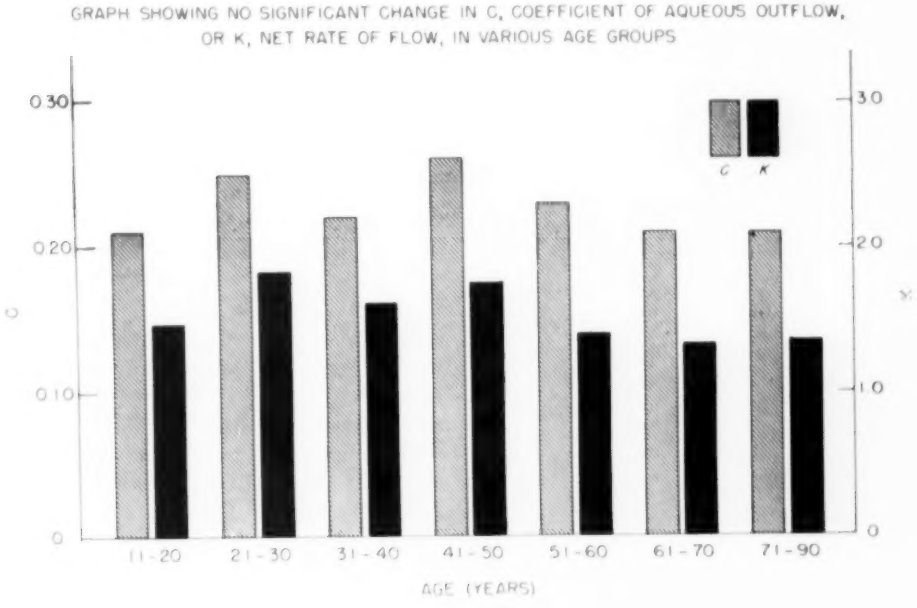

Figure 7

We performed tonography on a series of patients with normal eyes from the age of 11 to 90 years. Outflow studies were performed on 20 normal eyes in each decade from age 11 to age 70 years and on 20 normal eyes in the patients from 70 to 90 years of age. These tracings were all performed between the hours of 8:30 and 10:30 a. m. Most of the patients had been examined in the Ophthalmology Clinic at the Hospital of the University of Pennsylvania and found to have essentially normal eyes. Table 7 shows the years of age. The greatest variation between the average C value in any two decades was 0.04 cu. mm/min/mm. pressure, and the greatest variation in the K value was 0.51 cu. mm/min.

COMMENT

This paper discusses some of the technical difficulties which might be encountered by anyone in setting up apparatus for tonography and some precautions which should be observed. The results of a series of control studies on normal eyes are presented. Strict

attention to many details is essential for obtaining reliable and consistent tonographic tracings. The more important among these are a comfortable position for the patient in a quiet room, adequate topical anesthesia, satisfactory retraction of the eyelids, correct application of the tonometer, and good fixation.

Too large or too small a voltage output by the Mueller electronic tonometer can cause great difficulty. Ideally, a Mueller electronic tonometer should have a voltage output of very close to 250 mv., with a panel reading of 7, for use with the Sanborn recording system. If the voltage output of the tonometer is too high, a jack condenser can be inserted. If the voltage output is too low, changes can be made in the resistor in the output circuit of the tonometer. We attempted to determine the accuracy and reproducibility of the methods in our hands when used on normal eyes.

Studies were performed to determine the amount of variation in tonographic measurements at various intervals within a 30-hour period. A range of variation from the mean *C* of ±8% to ±30% was observed in this study, with an average variation from the mean *C* of ±0.045 cu. mm/min/mm. pressure, or ±19%. In a similar study, tonographic values obtained on normal eyes at similar intervals on five different days showed a range of variation from the mean *C* of ±16% to ±35%, with a slightly greater average variation from the mean *C* of ±0.053 cu. mm/min/mm. pressure, or ±25%. Both of these studies showed a lack of consistent diurnal variation in tonographic values and intraocular tensions in normal eyes. The results compare in general with those of Kronfeld,[5] in which he found a coefficient of aqueous outflow consistent within ±25% of the arithmetic mean in normal eyes at different times of the day.

We found an average variation in the *C* value on aqueous outflow tracings repeated at the same time of the day of 0.036 cu. mm/min/mm. pressure, or ±16.5%, with a range of variation from 0 to 0.10 cu. mm/min/mm. pressure. These results compare with Kronfeld's [5] findings when he reported a consistency of the coefficient of outflow within the limits of ±10% of the arithmetic mean.

In studying the distribution of the coefficient of aqueous outflow, we found that 31% of our 140 patients had a *C* value between the range of 0.11 and 0.19 cu. mm/min/mm. pressure, as compared with 35% in this range in Kronfeld's [5] studies. A range of 0.11 to 0.42 cu. mm/min/mm. pressure was found for the coefficient of aqueous outflow in normal eyes. When the average variation of *C* of ±16.5% to ±25% is applied to any of the 31% of *C* values in the range of 0.11 to 0.19 cu. mm/min/mm. pressure, an abnormal or borderline result may be obtained.

In studies on normal eyes in different decades of life, we found very little variation in the facility of outflow or net rate of flow. There is some slight decrease in the facility of aqueous outflow and the net rate of flow in patients past the age of 50. However, this did not appear great enough to be significant. The greatest variation between the average *C* value in any two decades was 0.04 cu. mm/min/mm. pressure, and the greatest variation in *K* value was 0.51 cu. mm/min. The average facility of aqueous outflow for patients over the age of 50 was 0.21 cu. mm/min/mm. pressure, and the average *C* for patients under the age of 50 was 0.23 cu. mm/min/mm. pressure.

We attempted to determine the need of a Sanborn recorder tracing for tonographic studies and found that such a record was very useful in occasional tonographic tracings that were extremely irregular. Otherwise, fairly reliable *C* values could be obtained by taking direct readings from the Mueller tonometer scale. In 240 tracings on 140 normal eyes, we found an over-all average *C* value of 0.224 cu. mm/min/mm. pressure and a *K* value of 1.45 cu. mm/min., which compared quite closely with those of other authors.

SUMMARY

Common pitfalls and possible sources of error encountered by the beginner in tonography are discussed.

No significant diurnal pattern of *C* or *K* values was found in normal eyes.

No significant change in *C* or *K* values was found in various age groups.

The average variation in *C* values when tracings were repeated on the same normal eye at different times of the same day was 0.045 cu. mm/min/mm. pressure, and when done on succeeding days it was 0.053 cu. mm/min/mm. pressure. When tracings were done at the same time on succeeding days, the average variation fell to 0.036 cu. mm/min/mm. pressure.

The extreme range in *C* values determined by repeating the tracing at the same time on succeeding days was 0.10 cu. mm/min/mm. pressure.

A probable error of approximately ±0.04 cu. mm/min/mm. pressure must be assumed for any one tracing on a given eye. This figure varies from 0.36 to 0.53 cu. mm/min/mm. pressure, depending on the method of calculating it.

The *C* value was 0.16 cu. mm/min/mm. pressure or more in 94% of 140 normal eyes. We feel that any value below 0.16 cu. mm/min/mm. pressure should be regarded as suspicious.

Dr. Morton W. Grant helped us in overcoming many of the technical difficulties encountered in setting up our apparatus and permitted us to visit his laboratory, where we observed his work and were instructed by him.

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Polysaccharides of the Vitreous Fibers

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The consistency and viscosity of the vitreous body seem to depend to a large extent upon the structure of the fibrous network formed by the so-called residual protein, which, on the basis of most recent evidence, can be assumed to be represented by fibers of collagen or material closely related to collagen.* Recent studies by various authors have shown that the formation and physiochemical properties of collagen fibers can be significantly influenced by certain strongly polarized substances, among which is an acid mucoprotein found in blood plasma.[3] The carbohydrate moiety of this mucoprotein shows essentially the same composition as the neutral mucopolysaccharides attached to all serum plasma globulin,[4] and the acid nature of the mucoprotein resides with the protein moiety.

It has been found by several authors that the isolated fibrous material of the vitreous contains a significant amount of carbohydrate bound to the protein of the fiber.[5] As the composition of this polysaccharide was not known, we determined it in the fibrous material obtained from the central part of the vitreous. This part was chosen in order to prevent extraneous material, particularly pigments from adhering to the surface of the vitreous. The present report deals with these experiments.

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* References 1 and 2.

EXPERIMENTAL METHODS

I. Preparation of Crude Fibrous Material from Central Part of Vitreous

Beef eyes obtained from the slaughterhouse immediately after the animal was slaughtered were cleaned from the adhering muscles immediately upon arrival and then stored overnight in a frozen condition at —15 C. They were then put into warm water until the superficial parts of the globe thawed out. These were separated from the more central part of the frozen vitreous. The latter was then thawed out completely and material pooled from 30 eyes. The pooled material thus obtained was homogenized in a glass homogenizer and centrifuged at 15,000 rpm at 0 C, the supernatant decanted, and the centrifugate suspended in about 10 cc. of distilled water and rehomogenized for five minutes under cooling with ice with a Virtis homogenizer. This procedure led to a separation of fibers in two layers, one floating on the surface and the other remaining on the bottom of the centrifuge tube. The two layers were then separated and twice rehomogenized and resuspended in fresh distilled water. The material which floated on the surface after the first homogenization now remained on the bottom of the centrifuge tube, while the material which remained on the bottom after the first homogenization did not produce any more floating material. The two fractions of the fibrous material were then stored frozen at —15 C until a sufficient amount of the material corresponding to the vitreous of 150 beef eyes was accumulated. The pooled material obtained from 150 eyes was then washed five times with large amounts of distilled water and centrifuged at 15,000 rpm in a refrigerated high-speed centrifuge.

II. Fractionation of the Fibrous Material of the Vitreous with Trichloroacetic Acid and Sodium Hydroxide

A crude fibrous preparation obtained as described above consists of two very different fractions. The main bulk, corresponding to about 90% of the total nitrogen of the crude fraction, is soluble in 5%

trichloroacetic acid (TCA) at 90 C, while the remaining 10%, which shows a brown coloration, is completely insoluble under these conditions, even after prolonged extraction. The soluble material is completely extracted by 5% TCA at 90 C after 20 minutes, and a longer extraction does not increase significantly the nitrogen content of the solution. The insoluble, colored, material may at least partly be due to the contamination of the crude fibrous preparation by small amounts of material from the pigmented elements of the ciliary body. The residue, which is not soluble in TCA, could be separated again in two fractions, which both contain polysaccharides attached to protein but differ in their composition. One of the two fractions of the TCA-insoluble residue was obtained by extracting the residue washed previously with 5% TCA with 1 N NaOH for 10 minutes at room temperature. This fraction will be designated as TCA-insoluble Fraction I. The residue of this extraction did not yield any further sugar, even after heating for 10 minutes at 100 C with 1 N NaOH. After it was heated for two hours at 100 C with 2 N H_2SO_4, the residue dissolved almost completely, and the solution contained a considerable amount of hexose. This fraction of the polysaccharide of the vitreous will be designated as TCA-insoluble Fraction II.

III. Analytical Methods

Nitrogen was determined by the micro-Kjeldahl method. Hexoses were determined by the three reactions of cysteine and sulfuric acid, previously described [8] under the assumption that no other hexoses but mannose, galactose, and glucose are present. In these three reactions the three sugars differ considerably with respect to their extinction coefficients, so that with use of the same standard three independent equations can be obtained by three determinations, from which the concentrations of all three sugars can be calculated. The presence of keto sugars was excluded by the cysteine carbazole reaction,[9] and the presence of pentose,[10] by the so-called basic cysteine reaction. Methylpentose [11] was determined by its cysteine H_2SO_4 reaction; hexosamine, by the indole HCl reaction.[12] For the latter determination the TCA extract and Fraction I were hydrolyzed for two hours at 100 C with 2 N HCl under reflux to liberate all hexosamine from its glycosidic linkages. The measurements of the optical density with samples of the TCA-soluble fraction were carried out as usual. The TCA-insoluble Fraction II, however, contained an unidentified substance which gave an intensive brown color with the indole HCl reagent, interfering with the measurement of the color developed by hexosamine. This interfering color product was removed completely by shaking the sample with 3 cc. of $CHCl_3$, and the color due to hexosamine was then measured in the water phase against a blank and a standard treated in the same way. Hexuronic acids were determined by two modifications of the carbazole reaction.† Because of the high content in protein and hexose, the determination of hexuronic acid was carried out in such a way that, in addition to the samples themselves, internal standards of glucuronic acid and of a solution of hexose containing as much galactose and glucose as was found in the TCA extract of fibers were run simultaneously. Controls, furthermore, were run for the samples, the internal standards, and the hexose standard, which were treated in exactly the same way as the experimental samples except that, instead of carbazole, pure ethanol was added. The optical density of every control sample was substracted from the corresponding experimental sample. This operation yielded the optical density of the reaction with carbazole. From the total optical density due to carbazole reaction in the experimental sample was subtracted the optical density due to the hexose in this sample. The difference represented the optical density due to hexuronic acid.

For further tentative identification of the constituents of the fiber polysaccharide, the fermentation test and paper chromatography were used. To this end, TCA had to be removed from the TCA extract. This was done by heating the extract first for 90 minutes at 100 C under reflux, then bringing it up with 10 N H_2SO_4 to 2 N, and heating again for two hours. This procedure hydrolyzed the polysaccharide and destroyed completely the TCA. Fractions I and II, containing no TCA, were directly hydrolyzed for two hours at 100 C with 1 N H_2SO_4. The sulfuric acid was then removed as barium sulfate and the hydrolysate concentrated in vacuo to a volume corresponding to about one-hundredth of the original volume of the vitreous from which the fibrous material was obtained. The fermentation test was carried out by mixing the hydrolysate with an equal volume of a 2% freshly prepared suspension of washed Fleischmann's yeast and shaking at room temperature for 20 minutes and for 2 hours. It was shown in preliminary experiments that under these conditions glucose was quantitatively removed from the solution after 20 minutes, whereas mannose and fructose required more than 2 hours for complete fermentation. Galactose was not removed at all (Table 1). Through this differential behavior, hexose may be identified and quantitatively estimated in two different ways. In the first procedure, the total hexose is determined in the original, nonfermented, sample and in the sample fermented for three hours by the so-called primary cysteine reaction and the cysteine reaction of mannose against a standard of mannose.[8] As

† References 13 and 14.

TABLE 1.—*Differences in Rate of Fermentation of Various Monosaccharides and Oligosaccharides by Washed Fleischmann's Yeast**

Exper. No.	Substrate	Values, in Mg./100 Cc. of Solution				
		Concentration at Time 0	Concentration After 20 Min. Incubation	Decrease, %	Concentration After 2 Min. Incubation	Decrease, %
1	Glucose	3.2	0.2	94	...	..
	Mannose	8.0	5.6	30	1.2	85
	Glucose + mannose	3.2	0.2	..	...	..
		8.0	4.9	39	...	..
2	Mannose	8.0	...	..	0.9	89
	Galactose	8.0	...	..	8.2	0
	Mannose + galactose	8.0	...	..	0.8	90
		8.0	...	..	8.0	..
3	Fructose	4.0	2.7	25	0	100
	Maltose	4.0	3.3	17	0.35	91

* 1.4 cc. of solution + 0.5 cc. of a suspension of 2 gm. of yeast in 25 cc. of H_2O + 0.3 cc. of H_2O.

galactose is not affected by yeast, these two determinations give two equations from which the amounts of glucose and mannose fermented by the yeast can be computed. The results of this determination are then confirmed by determining by the primary cysteine reaction the amount of sugar fermented in the time interval between 20 and 180 minutes. This amount is compared with the amount ing method. The amount of hexose remaining after fermentation is that of galactose. Any sugar removed by yeast in the time interval between 20 and 180 minutes is either D-mannose or D-fructose. The latter can be easily evaluated by the cysteine carbazole reaction of keto sugars. The paper chromatography, according to Bryson and Mitchell [15] and 180 minutes is either D-mannose or D-fructose. The latter can be easily evaluated by the cysteine carbazole reaction of keto sugars. The paper chromatography, according to Bryson and Mitchell [15] and Chargaff and associates,[16] could be carried out only on the TCA-soluble fractions. For this purpose, the above hydrolysate was still further concentrated in vacuo over sulfuric acid until the total amount of sugar approximately corresponded to 5 mg/cc. of the concentrate.

RESULTS

I. Composition of TCA-Extractable Polysaccharides of the Fibers

The saccharides present in the TCA extract of the fibrous material are resistant to hot 0.5 N NaOH and are, therefore, present in the form of a polysaccharide, or of oligosaccharides, the aldehydic end-group of which is combined with the proteins. They were originally precipitated by 5% TCA; with the proteins, they are therefore linked to the latter. When TCA extract is neutralized and dialyzed for 24 hours against running water, and then against distilled water at 4 C, with frequent change of water, a fibrous, gelatinous material precipitates, which is soluble in diluted salt solution and contains all the polysaccharides originally present in the TCA extract. This shows that the treatment with TCA at 90 C did not split the linkage between the carbohydrate and the fibrous protein. Three classes of sugars were found to be constitutents of these polysaccharides, namely, hexoses, which are the predominant constituents, hexuronic acid, and hexosamine. The test for methylpentose was always slightly positive, but the amount of the sugar was about at the limit which can be safely detected and determined by our analytic procedures; it was impossible, therefore, to confirm the presence of this class of sugars by other methods.

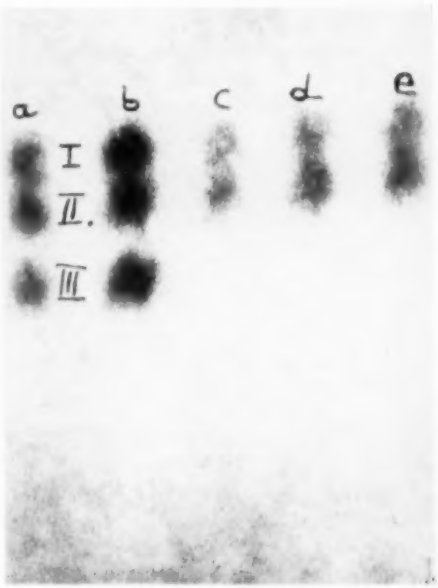
(*a*) *Hexoses.*—(1) Quantitative Determination: Only two hexoses, namely, glucose and galactose, were found to be present in significant amounts attached to the fibers. The quantitative determination, therefore, required only the use of two different color reactions. The so-called primary and secondary cysteine reactions were used for this purpose.[8] The determinations were carried out on the material which floated, after

homogenization, on the surface of the liquid, as well as material collected from the bottom. The first material was found somewhat purer than the latter. There was, however, no significant difference between the compositions of the polysaccharide in the two fractions of the fibrous material. The results of determinations of hexose listed in Table 2, therefore, represent sums of the values obtained for the two fractions. As can be seen, the ratio of galactose to glucose varied between 0.82 and 1.0, with an average of 0.94. The variations are within the limit of error of the method, which is relatively high in mixtures containing about equal amounts of glucose and galactose.

(2) Identification of Galactose and Glucose in the Polysaccharide by the Fermentation Test: The identification by fermentation was carried out on a hydrolysate prepared as stated above. The fermentation was carried out for 20 minutes and 3 hours. The amount and nature of the sugar which disappeared and remained in solution were then determined by the two above-mentioned cysteine reactions. The test was carried out on the material obtained from Experiment 6 of Table 2. Of the total absorption found in the solution in the primary cysteine reaction of hexose, 71% disappeared after 20 minutes' incubation, and 29% remained unchanged even after 3 hours' incubation. This residue behaved in the two cysteine H_2SO_4 reactions like galactose. No mannose, therefore, could have been present in solution. The glucose which disappeared represented 55% of the total hexose in solution, in good agreement with the analytical data listed for Experiment 6 in Table 2.

(3) Identification of Hexose by Paper Chromatography: The paper chromatography of the hydrolysate of the polysaccharide with butanol-pyridine-water mixture, according to Bryson and Mitchell [15] and to Chargaff and associates,[16] yielded two spots identical with the spots of glucose and galactose, as can be seen in the accompanying Figure. A third spot, with a much lower R_F‡ was visible only in the ultraviolet portion of the spectrum and could correspond to hexosamine. No other indication of a third hexose was found, even when the amount of hexose used in the sample for chromatography was as high as 100γ.

(4) Fractionated Extraction of Hexoses of the Fibers by TCA: To ascertain whether glucose and galactose of the TCA-soluble fibers are combined with each other or with the fibrous protein in a fixed ratio, a fractionated extraction of the fibers with TCA



Paper chromatogram of concentrates of hydrolysates of vitreous fibers. Spots *a* and *b*: standards of (*I*) galactose, (*II*) glucose, (*III*) mannose (10γ and 20γ). Spots *c*, *d*, and *e*: three concentrations of the fiber hydrolysate.

was carried out. To this end, the fibers were first extracted for only 5 minutes at 90 C with 5% TCA, then washed in the cold with TCA, and again extracted for 15 minutes. A third extraction, for 20 minutes, yielded no more appreciable amounts of hexose. Glucose and galactose were then determined in the two extracts. The results

‡ R_F is proportion of distances of the starting point from adsorbate and from the solvent front.

of three such experiments are listed in Table 2. Whereas in the first experiment the ratio of galactose to glucose in the two extracts was almost identical, in the two others there was a slight deviation. This deviation, however, in Experiment 3, was in the direction opposite to that in Experiment 2, and the deviations fall within the limit of error of the method, which is particularly high when the concentrations of galactose and glucose are almost identical, as was the case in our experiments. These results, therefore, indicate that glucose and galactose are either constituents of one single polysaccharide or that two different polysaccharides are combined with the fiber proteins in identical ratios. The fibers go into solution as a whole, as indicated by the ratio of nitrogen to hexose.

TABLE 2.—*Composition of Polysaccharide Preparations Obtained by Fractionated Extraction of Fibers with 5% TCA at 90 C*

Exper. No.	Time Interval of Extraction, Min.	Galactose and Glucose, Mg./100 Mg. N		Ratio Galactose / Glucose	Hexuronic Acid As Glucuronic Acid, Mg./100 Mg. N	Non-polyuronide Hexosamine, Mg./100 Mg. N	Fiber Nitrogen, Mg. in 100 Cc. Vitreous
11	0-5	17.5	16.8	1.04	0	....	0.28
	5-20	21.0	15.4	1.36	....	....	0.49
12	0-5	18.3	19.2	0.95	0	1.72	0.23
	5-20	17.5	17.0	1.03	2.76	1.84	0.56
6	0-20	16.4	18.6	0.88	3.64	2.32	1.16

(*b*) *Hexuronic Acid.*—The crude fibers separated from the vitreous and washed on the centrifuge contained considerable amounts of hexuronic acid, detectable by the carbazole reaction. Most of it, however, could be removed by repeated homogenization in a mechanical Virtis homogenizer and subsequent washing of the fibers with H_2O. After threefold homogenization at about 25,000 rpm for five minutes, a certain residual amount of hexuronic acid remains, which cannot be removed by further homogenization and washing. The hexuronic acid in the crude preparation reacts, in the modification of the carbazole reaction, differentiating between various mucopolysaccharides, such as hyaluronic acid.[14] It seemed possible that the residual hexuronic acid in the homogenized fibers represents hyaluronic acid trapped in the meshes of a very fine network. An attempt, therefore, was made to remove this residual hyaluronic acid by hyaluronidase. In one experiment of this series, the homogenized and centrifuged fibers, prior to TCA extraction, were suspended in 10 cc. of a 0.1 M acetate buffer, of pH 5, containing 0.2 mg/cc. of a hyaluronidase preparation of about 1000 units/mg. from bull testis and shaken in the presence of toluene at room temperature for 24 hours. The fibers were then centrifuged off and the supernatant decanted. The centrifuged fibers were washed with the buffer and resuspended in the same amount of buffer, this time containing 1 mg/cc. of enzyme, and again shaken for 24 hours under toluene. The supernatant was again separated from the fibers by centrifugation and the treatment with the enzymes repeated once more. The centrifuged fibers were then repeatedly washed with water, suspended in 5% TCA, and extracted for 20 minutes at 90 C, as usual. The two supernatants were evaporated to dryness over P_2O_5 in vacuo and redissolved in 5 cc. of water. This procedure was carried out with the two fractions of fibers which separated during the original centrifugation into bottom and surface layers, individually. Determination of hexuronic acid by the carbazole (and hexose by the cysteine H_2SO_4) reaction was then carried out on supernatant fluid of the hyaluronidase-treated fibers, as well as on the TCA extract of fibers. The surface-layer fibers after this treatment in Experiment 9 contained amounts of hexuronic acid corresponding to no more than 3% of the total carbo-

hydrate. The bottom layer had somewhat more, corresponding to 7% of the total carbohydrate. In fibers not pretreated with hyaluronidase the amounts of hexuronic acid represented about 20% of the total carbohydrate. The supernatant of the hyaluronidase extraction contained no hexose whatsoever but considerable amounts of hexuronic acid. It is clear, therefore, that at least most of the polyuronide of the fibers which could not be removed by homogenization and washing can be separated from the other sugars of the fibers by hyaluronidase and can be assumed, therefore, not to be combined chemically with hexoses. This conclusion is borne out by the results of hexuronic acid determination in TCA solution of fibers obtained by fractionated extraction, described above and listed in Table 2. As can be seen, the five-minute extract, which contained 30% to 40% of the total hexose of the fibers, did not contain any significant amounts of hexuronic acid. This, however, was completely extracted during the following 15 minutes.

(*c*) *Hexosamine.*—Hexosamine was determined by the indole HCl reaction after a two-hour hydrolysis at 100 C with 2 N HCl. This time of hydrolysis was chosen on the assumption that the hexosamine found in excess of that derived from hexuronides is hydrolyzed off, as was found for the so-called neutral mucopolysaccharides of blood group substances and neutral mucopolysaccharides of body fluids. To determine the amount of this nonpolyuronide hexosamine, it was necessary to subtract from the total hexosamine found the amount present in the polyuronides. The latter were calculated on the assumption that 1 mole of glucosamine is combined with 1 mole of hexuronic acid. It has previously been found that the amount of hexosamine liberated from acid mucopolysaccharides, such as hyaluronic acid and chondroitin sulfuric acid, by 2 N HCl after two hours at 100 C corresponds in the indole HCl reaction to 55% of the theoretical. It was possible, therefore, to calculate from the known amount of hexuronic acid, which could be assumed to be identical with glucuronic acid, the amount of hexosamine in the polyuronide fraction. The values for the total and nonpolyuronide hexosamine are listed in Table 2. As can be seen, there is a significant amount of the latter in the TCA extract of fibers, which corresponds to about 8% of the total hexose. The validity of this result is borne out by the determination of hexosamine in the five-minute TCA extracts of fibers in Experiment 12 of Table 2, where 1.72 mg. of hexosamine per 100 mg. of nitrogen was found in absence of any hexuronic acid and by the presence of hexosamine in fibers of the surface layer pretreated with hyaluronidase in Experiment 9, which contained only barely significant amounts of hexuronic acid.

2. The Non-TCA Extractable Material

(*a*) *Fraction II.*—This fraction is represented by material which is not extracted at 90 C, even by prolonged incubation with TCA, but goes into solution after 10 minutes' incubation in 1 N NaOH at room temperature. The protein content of this fraction represents only 5% to 6% of that of the TCA-soluble fraction (Table 3). This protein is combined with a polysaccharide of a completely different type from that of TCA-soluble Fraction I. As can be seen in Table 3, this polysaccharide contains in every case at least two hexoses, namely, galactose and mannose, in approximately equivalent amounts. These two hexoses were identified by their extinction coefficients in the primary and secondary cysteine H_2SO_4 reactions and by fermentation tests after two hours' hydrolysis with 2 N H_2SO_4. In some samples there was also a certain amount of glucose present, identified by the fermentation test. The latter, however, did not exceed 20% of the total hexose, and its absence, in some cases, indicates that it probably was the result of contamination with another fraction. In addition to hexoses, the polysaccharide contains hexosamine, in amounts about equal to those of mannose, and some methylpentose, the amount of

TABLE 3.—*Comparison of the Polysaccharides from the TCA-Insoluble Fractions II and III of Vitreous Fibers*

(All values expressed in micrograms per 100 cc. vitreous)

Exper. No.	Fraction	Glucose	Galactose	Mannose	Total Hexose as Galactose-Mannose	Fucose	Hexosamine	Ratio: Fucose/ Galactose-Mannose	N
16	II	....	....	....	16.2	1.0	...	0.062	36
	III	47	....	....	....	...	...	.....	..
16	II	2.9	12.9	12.5	....	2.80	8.7	0.110	..
	III	9.7	....	....	....	...	...	.....	..
17	II	....	....	....	18.6	1.74	...	0.093	..
	III	23.4	....	....	....	...	...	.....	4.7

which in various preparations varied between 7% and 9% of the total hexose. The ratio of the total hexose to nitrogen in this fraction was about 3:10, and therefore it was not very different from that of Fraction I. The polysaccharide of Fraction II is a mucopolysaccharide of the type found widely distributed in animal tissues and very similar in its composition to the mucopolysaccharide in the so-called mucoid I found in combination with the globulins of the human plasma.[4]

The similarity in composition between the mucopolysaccharide of Fraction II and the mucopolysaccharide attached to the soluble proteins of the vitreous suggested the possibility that the whole Fraction II may be the result of a contamination of the fibrous material of the vitreous with a soluble protein which is so labile that it becomes insoluble by the freezing of the whole vitreous in the deep freeze. The so-called β-lipoprotein of the human blood plasma is known to be denatured and insolubilized by freezing below —15 C. To test this possibility, control experiments were carried out in which the vitreous was obtained not frozen, but fresh, and immediately centrifuged and the insoluble residue washed immediately, first with saline and then with distilled water. The washed fibers were then frozen overnight and the next day processed as usual. It was found that after 20 minutes' extraction at 90 C with 5% TCA, the carbohydrate containing Fraction II was no longer present. The presence, therefore, of mucopolysaccharides of Fraction II, previously obtained from frozen vitreous, was due to a denaturation of a glycoprotein extractable with saline. Our glucoprotein of Fraction II, therefore, could be an artifact not related to the fibrous material of the vitreous. It was possible, however, that the glycoprotein of Fraction II is in vivo absorbed on the fibrous material of the vitreous and, while it can be removed from the fibers by washing with saline before freezing, it becomes denatured and firmly attached to the fibers by the freezing process. To decide this question, the supernatant of the fresh vitreous from which the fibers were removed by centrifugation was stored overnight at —15 C, thawed, and centrifuged at the same speed as was used the previous day for the separation of the fibrous material.

A barely perceptible precipitate appeared, which, after decantation of the supernatant, was suspended in 5% TCA, centrifuged, and extracted with 5% TCA at 90 C for 20 minutes; the insoluble residue was then dissolved in 1.5 cc. of 1 N NaOH. This precipitate did not contain any appreciable amounts of carbohydrate. The possibility still remained that particles of denatured glycoprotein which could not be spun down by themselves could be pulled down together with the fibers or be absorbed on the latter and the two thus come down together during centrifugation. To test this possibility, 300 cc. of fresh, nonfrozen vitreous was centrifuged to separate the fibrous material. The supernatant, free of fibers, was stored in the deep freeze overnight. The next day 300 cc. of vitreous was prepared from fresh, nonfrozen eyes. The fibers coming from fresh vitreous and the frozen vitreous,

free of fibers, were then combined and centrifuged at 12,000 rpm in the Spinco ultracentrifuge. The fibers so obtained from the combined vitreous were washed with saline and distilled water, stored overnight in the deep freeze, and the next day homogenized, washed, and extracted at 90 C with 5% TCA, as usual. The TCA insoluble residue was extracted for 10 minutes at room temperature in 1 N NaOH. This solution, which would correspond to the Fraction II from 300 cc. of frozen vitreous, contained altogether 45γ of a hexose and about 1γ of methylpentose. This experiment shows that freezing of the vitreous produced a denatured glycoprotein which can attach itself mechanically or by adsorption. The amount of this material, however, which was precipitated in our experiments was one-half to one-fourth the amount of carbohydrate that was found in three of our experiments in Fraction II.

(*b*) *Fraction III.*—After complete extraction of the carbohydrate soluble in NaOH at room temperature, another carbohydrate fraction can be obtained from the insoluble residue by two hours' hydrolysis in boiling 2 N H_2SO_4. This hydrolysis brings almost all of the residue in solution and yields a sugar which, in our fermentation test, was completely fermented in 20 minutes at room temperature. The insoluble polysaccharide, therefore, consists exclusively of D-glucose. In addition to glucose, the hydrolysate contains a nitrogenous substance (Table 3). On the assumption that the latter is a protein, it can be calculated from the nitrogen content of the hydrolysate that it contains about as much protein as glucose. The Fraction III, therefore, contains a glucosan which is probably combined with a protein that is insoluble in TCA and NaOH. It also seems possible that the glucosan, in itself, may be soluble but is surrounded by an insoluble protein membrane, which prevents it from going into solution. As the insoluble residue of the particulate material of the vitreous always contains black pigment, in varying amounts, the possibility had to be considered that Fraction III is derived from material in some way combined with the pigment particles. To test this, a large amount of pigment which had been removed during the preparation of the vitreous was treated exactly in the same way as the particulate material of the vitreous. The final hydrolysis of the residue of the pigment remaining after extraction with 5% TCA at 90 C and NaOH at room temperature yielded a sugar which behaved in the same way as that of Fraction III. The latter, therefore, is most probably the result of the contamination by extraneous pigment or is derived from small pigment particles which may normally be present in the vitreous.

COMMENT

Our experiments indicate that the particulate material from the central part of the vitreous consists of three protein fractions, each associated with a different carbohydrate. The predominant fraction is the fibrous material extractable with TCA. The two other fractions differ, apart from the composition of the accompanying carbohydrate moiety, by their solubility in alkali and by the ratio between protein and carbohydrate. They seem to be the result of contamination by extraneous material or by denatured material originally present as soluble proteins of the vitreous.

The TCA-extractable fibers themselves contain one or two polysaccharides firmly combined with the protein. This polysaccharide is not esterified with H_2SO_4, since after 20 hours' hydrolysis of the 5-minute TCA extraction of fibers with 5.8 N HCl, with following adjustment to pH 2.5 and the addition of $BaCl_2$, no precipitate of barium sulfate was obtained. These neutral polysaccharides, in addition, appear associated with the polyuronide which, on the basis of its behavior toward hyaluronidase and in the reactions with carbazole and H_2SO_4, seems to be identical with or related to hyaluronic acid. The fact that this poly-

uronide can be almost completely removed by treatment with hyaluronidase without bringing any hexose into solution shows that there is no covalent chemical bond between the hexuronic acid and the neutral polysaccharide of the fibers. It seems most probable that this polyuronide represents hyaluronic acid trapped in a very fine fibrous network. This possibility is borne out by the fact that a five-minute extraction with TCA at 90 C of the fibrous material, while bringing into solution 30% to 40% of the hexose and hexosamine combined with it, does not extract any significant amounts of hyaluronic acid, which is then extracted completely after the 15-minute extraction. It seems reasonable that individual fibers or fibers on the surface of chunks of fibrous material will first be extracted, while fibers located more in the center require a longer extraction. It is obvious that if hyaluronic acid is trapped in meshes of a fibrillary network, it will be liberated only after the centrally located fibers are dissolved. This situation also explains the very slow action of hyaluronidase, since, obviously, the enzyme can only slowly penetrate into the narrow meshes of such a network, filled with a very viscous material. It is, of course, possible that this network represents an ultramicroscopic structure.

The main bulk, however, of the saccharides attached to the TCA-extractable fiber is represented by one or two neutral polysaccharides. The fact that the amount of hexosamine is very much lower than the amount of hexose can be interpreted in two ways. Hexosamine could be combined with two hexoses, namely, galactose and glucose, which would be present in a molecular ratio of about 1:1, to an asymmetric molecule, consisting, for example, predominantly of a chain of disaccharide units of glucose and galactose, interrupted at certain intervals by a trisaccharide containing hexosamine in addition to hexose. Heteropolysaccharides with nonstoichiometric ratios between certain constituents are widely distributed in bacteria, plants, and animal tissues. Neutral mucopolysaccharides attached to serum proteins, for example, contain 0.7 mole of hexosamine and, depending on the species, 0.02 to 0.2 mole of fucose for 1 mole of hexose. In some heteropolysaccharides of this type one of the constituents can be present in much smaller amounts than the other. The lung galactan isolated by Wolfrom and associates[17] has recently been shown by antibody precipitation to contain at least one polysaccharide, consisting of galactose and glucuronic acid, the latter representing less than 10% of the total sugar. Another possibility, which appears more probable, would be that the fibers contain at least two neutral polysaccharides. One of these would be a mucopolysaccharide consisting of galactose and hexosamine only. If we assume that the mucopolysaccharide of the fibers has the same composition as that of the body fluids, which consist only of galactose and hexosamine, we can calculate from the amount of hexosamine that is not bound to uronic acid the amount of galactose present in the form of a neutral mucopolysaccharide. By subtracting this value from the total amount of galactose attached to the fibers, we obtain the amount of galactose combined with glucose. On the basis of our analytical data, the ratio of glucose to galactose in the latter would be 5:4.

A polysaccharide consisting of glucose and galactose in ratio of 2:3 has previously been found in the fibrous lens capsule of beef and rabbit.§ The polysaccharides of the lens capsule and that of the fiber of the vitreous seem closely related. These findings suggest the possibility that polysaccharides consisting solely or predominantly of galactose and glucose may represent a characteristic prosthetic group of the fibrous structures in animal tissue. The ratio between the different components of the polysaccharides could vary with the source of the fiber.

The mucopolysaccharide of Fraction II is, on the basis of our experiment, most probably due to a contamination by small amounts of a soluble protein in the vitreous

§ References 6 and 7.

which is denatured by freezing below —15 C. It seems interesting in this connection that Glegg and his associates‖ found in fibrous and connective tissue from various organs polysaccharides which on hydrolysis yielded galactose, mannose, glucose, and another sugar which, when isolated by paper chromatography, showed in certain organic solvents an R_F identical with that of fucose. The preparations of these authors represented either the residue from extraction of tissues with distilled water or extracts of the connective tissue with alkali at room temperature. This procedure of preparation corresponded to the methods used in our experiments for the preparation of Fraction II, and the question, therefore, arises whether the polysaccharides obtained by Glegg and his associates are not partly derived from glycoproteins or mucoproteins of the interfibrillary substance, which are either insoluble in distilled water and are precipitated during the extraction or are extracted with sodium hydroxide, like the mucopolysaccharides of our Fraction II. Under these circumstances, the polysaccharides of the fibers of connective tissue could represent a mixture of a polysaccharide consisting of galactose and glucose analogous to that found in the fibers of the vitreous and the mucopolysaccharides due to contamination from other sources. On the other hand, we must consider the possibility that the vitreous, like the fibers of the lens capsule, originates from ectodermal elements during embryonic development, and the glucogalactosans of these fibers, therefore, may be characteristic only for fibers of this origin.

SUMMARY

The particulate matter of the frozen and thawed-out vitreous consists up to 90% or 95% of a fibrous material that can be quantitatively extracted by 5% TCA at 90 C in 20 minutes.

These fibers are combined with one or several polysaccharides which contain glucose and galactose in nearly equivalent amounts, and much smaller amounts of an unidentified hexosamine.

In addition, a certain amount of a polyuronide is loosely attached to the fibrous material and can be separated from it by incubation with hyaluronidase.

The vitreous contains a small amount of a soluble glycoprotein which becomes insoluble by freezing at —15 C and is precipitated with the fibers.

The possible significance of these findings for the problem of the polysaccharide moiety of collagen fibers is discussed.

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Chorioretinitis Produced by Atomic Bomb Explosion

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In this atomic age it is necessary to disseminate all the facts that can be ascertained regarding the effects of atomic explosions. With this in mind, the following case report is presented.

REPORT OF A CASE

S. H., a white man 24 years of age, entered the Army in December, 1950. His entrance examination revealed vision as 20/20 in each eye. In August, 1952, he became a candidate for the Engineer Officers School, and the examination at this time showed 20/20 in each eye. He graduated from this school on Feb. 2, 1953. On April 25, 1953, as a Second Lieutenant, he was in charge of a platoon of soldiers detailed for training purposes at Yucca Flats, during an atomic explosion test. At 4:30 a. m., before sunrise, his men marched down a ramp into a trench 5 ft. deep. He was the last man to enter the trench. The tower from which the explosion was to take place was on his left, at a distance of about 3700 yd., and, as he heard the count of 3-2-1, he "sneaked" a look over his left shoulder, toward the tower, and at that moment the bomb exploded. He saw "a very bright, white, blinding light," and then continued walking into his trench. He was blinded for several minutes, so that he saw nothing. Then the men in the trench appeared like white shadows. All the color "round about was washed out." For the rest of that day his vision remained blurred. Seven hours later the vision in the right eye cleared up. On attempting to read, he noticed a spot on the page which moved with his eyes, and the print was distorted. He thought that this condition would clear up in time, but on the next day he reported to his medical officer, who transferred him to the hospital on the day following. He was at the hospital at Fort Hood, Texas, from April, 1953, to the end of May, 1953. He was separated from the service on June 2, 1953.

The hospital records of April 28, 1953, relative to the eye examination were as follows: "There is a large exudate in the retina with a small dark central spot. The color of a "hole" and/or hemorrhage is present. This spot is the size of the normal fovea. The white exudate is about one and one-fourth times the size of the optic disc. The retina does not appear to be raised, but the exudate appears raised about 1D. from the normal level of the retina. At the margins to the right of the mass vessels can be made out, but in the central portion they are obscured. A narrow rim of edema, about one-fourth the width of the mass, is present at the superior edge. The active white exudate is apparently three-fourths above and one-fourth below the macula. Inferior to it there are small areas of edema, and a superficial hemorrhage is present. This hemorrhage appears to be flat. The vision in this eye is 20/100."

The patient was placed on treatment with a salt-free diet, cortisone systemically, and atropine locally. A blood study done at that time showed a slight increase in white cells. On May 2, the area of white exudate decreased remarkably, being only one-half its original size, and persisting mostly in the area originally occupied by the superior half of the exudate. The inferior half showed a change in color, probably due to hemorrhage and pigmentation. Edema of the retina had subsided, and radiating tension lines were appearing.

On May 15, the previous white area appeared dark-yellow and light-brown. The fovea still showed what appeared to be a "hole," and it was darker in color than the rest of the retina. Pigmentation appeared inferior to the fovea. The central scotoma on May 26, with a 3 mm. white test object at 1 meter, measured 11.5 by 9 cm.

An examination made at the Brooklyn Regional Office of the Veterans Administration on April 18, 1955, was reported as follows: "Vision in the right eye is 20/20 and in the left eye 20/100. A refractive error, consisting of a +0.50 sph ⊐ +0.75 cyl, ax 85,

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is present in both eyes. The left eye cannot be improved with this correction. The lids and conjunctivae are normal in appearance. Both corneae are clear and transparent. The anterior chambers are of normal depth. No cells are seen in the anterior chamber. The iris markings appear normal. The left pupil is 4 mm. and the right 3 mm. in diameter. Slit-lamp examination reveals no opacity in the lens of either eye. The pupils dilated well with 10% phenylephrine (Neo-Synephrine). The vitreous is clear, and no floating opacities are seen. The fundus of the right eye is essentially normal. The left fundus shows a sharply outlined optic disc, of normal color. The retinal arterioles and veins are of normal size and caliber. In the macular area of the retina there is a pigmented area about one-fourth the disc diameter in size. The shape is roughly that of an inverted mushroom. An absolute scotoma is present within the 5-degree circle of the central field. The rest of the physical examination is essentially normal. The x-ray of the chest is negative; the Kahn test is negative, and x-rays of the sinuses are normal."

COMMENT

In January, 1955, Byrnes and co-workers [1] showed the results of exposing the eyes of rabbits to the initial explosion of the atomic bomb. On the basis of their prior computations on a 20 K.T. equivalent bomb, they concluded that in the unprotected human eye, at night, under exceptionally clear air conditions, the threshold for retinal burns, even to a distance of 40 miles, would be surpassed. They further stated that the pupil at night will admit 50 times as much light as the smaller pupil in the daytime, and concluded that the daytime hazard is very much reduced. They also stated that the fireball of the atomic bomb in its early phase is, roughly, 100 times as bright as the sun.

In the pamphlet, entitled "The Effects of Atomic Weapons," prepared by the U. S. Department of Defense and the U. S. Atomic Energy Commission, under the direction of the Los Alamos Scientific Laboratory,[2] it is stated that the effect of thermal radiation on the eyes is surprisingly small; even those who looked directly at the explosion at Hiroshima and Nagasaki, from some distance, of course, reported only temporary loss of vision. In one case a patient was so blinded by the flash that he was unable to distinguish light from dark for two days, but eventually his recovery was complete.

I assume that one of the reasons for so few thermal radiational effects on the eyes at these places was that the explosion was unexpected and that by the time the onlookers watched the fireball, the intense light had already passed to a great extent. In the present case reported, the knowledge of where to look prior to the explosion allowed the patient to see the original blinding flash of the detonation. The effect of this flash takes place in less time than the blink reflex.

SUMMARY

The energy released from an atomic bomb blast has visible infrared and long ultraviolet rays, which are capable of producing thermal burns involving the choroid and retina. The pupillary diameter at the time of detonation is a very important factor. The pupillary and blink reflexes are too slow to prevent burning of the retina because sufficient energy for this arrives after the first 0.1 second. These reflexes take a longer time. The distance at which burns may be produced is much greater than that at which any other harmful effects of the atomic bomb occur, and at a given distance the burns on the retina are severer than those of the skin. This is true because of the optical system of the eye. It seems apparent that in this case the right eye did not receive the rays because of the shielding effect of the bony structures of the nose. It is concluded that the necessity of covering the eyes during an atomic blast is of the utmost importance.

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Porencephaly

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Porencephaly is a relatively rare condition characterized by a defect or cavity in the brain which often communicates with the ventricular system and subarachnoid space and is filled with cerebrospinal fluid. Although the condition is well documented in the neurologic and roentgenologic literature, the ocular manifestations have been reported only rarely by ophthalmologists. Because of this, it was thought worth while to present the following review of the subject and unusual case report.

Even though this condition was known before 1859, it was not until then that Heschl * first applied the term porencephaly to 11 cases in which there was cavity formation in the brain substance communicating with the ventricular system and subarachnoid space. In the course of time, however, the concept of the term has changed, so that today many authors designate any cystic cavity in the brain as porencephaly except in the case of a cystic tumor.

ETIOLOGY

Most cases are first found at autopsy in children or young persons who had presented neurologic symptoms before death. These have been designated as congenital. Others appear after trauma and are called acquired. However, since no case of intrauterine development has been reported, the majority of authors feel it is incorrect to consider the condition as congenital.

Heschl [1] at first thought these cyst formations were all due to congenital maldevelopment. Later, however, after finding a case at autopsy with complete absence of pial vessels, he theorized that the vascular phenomenon was the underlying cause. Most authors now point to some circulatory disturbance, with subsequent ischemia and necrosis of the brain tissue, as the explanation for these cysts.

Penfield [2] has observed constriction of large pial arteries capable of arresting blood flow after convulsions in habitual epilepsy. He has postulated that porencephaly may result from severe localized vascular spasm or from cerebral thrombosis secondary to marked dehydration in children with acute febrile illnesses. Other vascular accidents may lead to this condition and have been produced experimentally by Evans and McEachern.[3]

LeCount and Semerak [4] feel that inflammation may lead to vascular embarrassment. Eisenstein and Taylor [5] and Globus [6] agree with this and believe that the commonest cause of porencephaly is intrauterine inflammation, with subsequent endarteritis and thrombosis of pial vessels leading to ischemia of brain tissue and eventual necrosis, glial proliferation, and cyst formation. Takahashi† points out the possible role of syphilitic arteritis as an underlying cause.

Many recent authors agree with Jaffé [8] that some form of trauma followed by hemorrhage and circulatory disturbance is the most important cause. He points out that the parietal region is especially exposed to birth injuries, and it is this area that is most commonly involved by porencephaly. In

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* Heschl,[1] cited by LeCount and Semerak [4] and Christensen and Schondel.[7]

† Takahashi, cited by Christensen and Schondel.[7]

Pendergrass and Perryman's[9] series, the parietal region was involved in 69%.

PATHOLOGY

Strom-Olsen,[10] Jaffé[8] and others have described the pathologic picture. The cyst wall may consist of an outer connective tissue layer and an inner layer of fibrillary glia. Exaggerated gliosis may appear, separating the cyst from the white matter, and this may lead to difficulty in differentiation from astrocytoma. In fact, in one of Pendergrass and Perryman's[9] cases, the neuropathologist could make this differentiation only on the basis of the clinical history.

DIAGNOSIS

The clinical findings, of course, depend upon the size and location of the defect. Small cysts may cause no symptoms and only be discovered at autopsy as a coincidental finding.

Patten, Grant, and Yaskin[11] describe a syndrome which they believe is clinically indicative of a porencephalic cyst. It is characterized by failure of development of one-half or part of one-half of the body, motor neuron weakness, sensory disturbances, and Jacksonian fits. Of Pendergrass and Perryman's[9] patients, 26 of the 29 first sought medical attention because of epileptic attacks, which had usually been preceded by such other neurologic symptoms as weakness of an extremity, strabismus, or mental retardation. In none of their cases were the eye findings the presenting complaint, but the following were noted: papilledema, in three patients; homonymous hemianopsia, in four; bitemporal hemianopsia, in one, and peripheral constriction of the visual fields, in one.

Before the introduction of pneumoencephalography and ventriculography, the diagnosis was made only post mortem. Now, however, if the subarachnoid space communicates with the ventricles, the diagnosis can be established during life by the pneumoencephalogram.

The differential diagnosis includes an incompletely filled dilated ventricle; a gliomatous, hydatid, or similar cyst, and an air-filled subdural hematoma. The specific differentiation of these entities lies in the realm of the roentgenologist and will not be

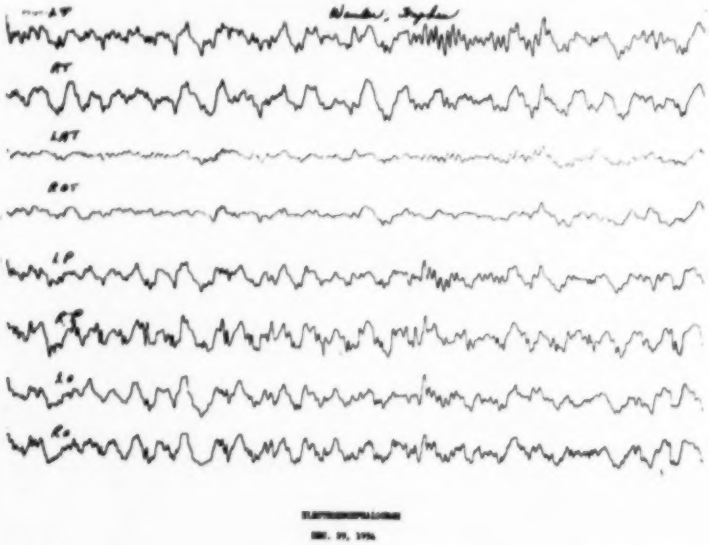


Fig. 1.—Electroencephalogram performed Dec. 29, 1954, showing an abnormal tracing consistent with a right temporoparietal focus.

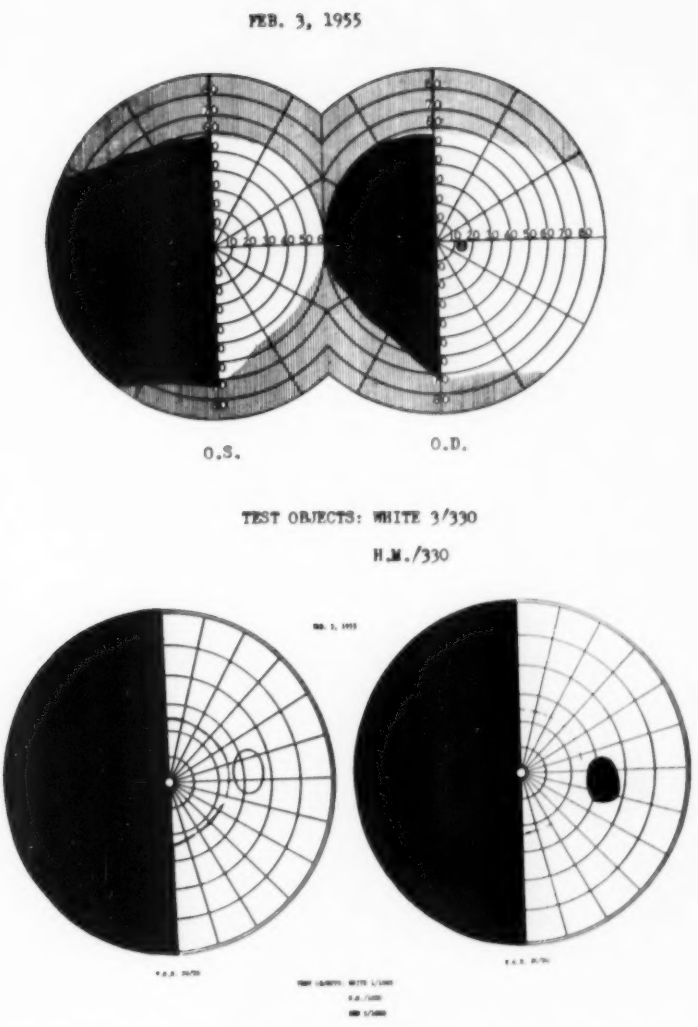


Fig. 2.—Peripheral and central visual fields, showing absolute congruous left homonymous hemianopsia with sparing of fixation on Feb. 3, 1955. Defect was present to all isopters.

discussed here, except to point out that a porencephalic cyst may simulate any intracranial mass lesion and can be differentiated preoperatively only by pneumoencephalography or ventriculography.

REPORT OF A CASE

S. W., a 12-year-old white boy, was referred for examination by his pediatrician on Feb. 3, 1955, because of severe, prostrating right frontal headaches for the past two weeks. For the previous four days there had been associated photophobia, low-grade fever, mild pharyngitis, slight meningismus, and a 1+ Kernig sign. In addition, for the past two weeks he had complained of having seen horses run from left to right across his field of vision. Penicillin had been given for four days, without relief of symptoms.

The past history revealed that the patient was a normal full-term baby without complicated or prolonged delivery. At the age of 2 years he had fallen, suffering a hematoma of the scalp with edema extending over the right temporal area. The

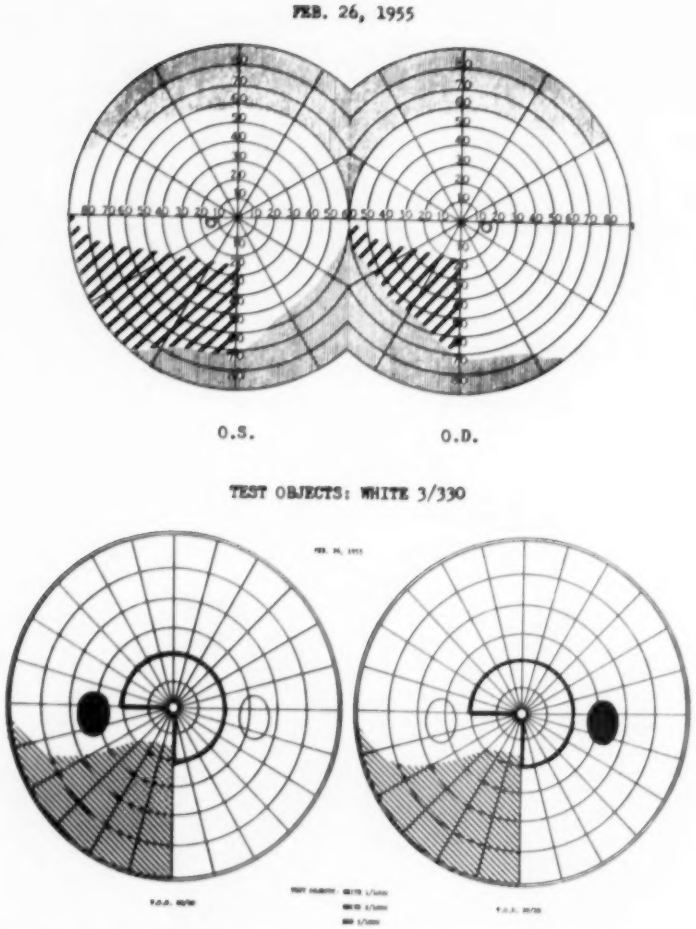


Fig. 3.—Peripheral and central visual fields showing relative congruous left lower quadrantonopia on Feb. 26, 1955, with defect present to white 3/330 test object on peripheral field and white 1/1000, white 2/1000, and red 5/1000 on central field. Fields were full to larger isopters.

physical examination at that time and the immediate follow-up were normal. When he was 3 years old, he had acute tonsillitis associated with high fever and generalized convulsions that lasted for several hours. After this, left-sided paresis of the extremities and trunk persisted for two weeks. He was apparently well after this until he was 8 years old, when he developed headaches and episodes suggestive of petit mal seizures. He was first seen in our office at that time, when the visual acuity was 20/20 in each eye and the examination normal except for simple myopic astigmatism. Reexamination in January, 1952, and October, 1953, revealed no significant change in the ocular examination. He was apparently well until Dec. 24, 1954, when he had a left-sided convulsion, which resulted in inability to move the left arm for a few minutes. On Dec. 25 he fell out of bed and had twitching of the left arm and leg again. On Dec. 27 he had had a "left-sided" convulsion. None of these attacks was associated with unconsciousness. Examination by a neurosurgeon on Dec. 27 was entirely negative except for use of his left hand, which was somewhat awkward, and the deep reflexes, which were more active in the left arm than in the right. Vision at that time was 20/20 in each eye, and gross confrontation fields, taken by the neurosurgeon, were full. X-rays of the skull were negative, and an electroencephalogram was reported as abnormal and "consistent with the

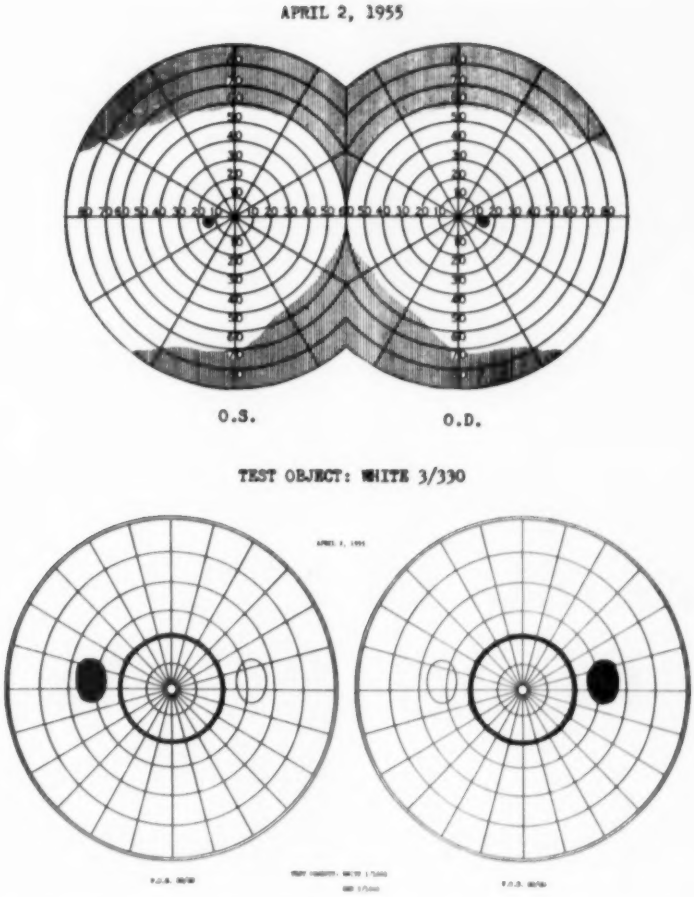


Fig. 4.—Normal peripheral and central visual fields, showing complete recovery on April 2, 1954.

diagnosis of convulsive disorder, right temporoparietal focus." The child was placed on phenobarbital therapy and was free of symptoms until the present illness.

Examination of the eyes on Feb. 3, 1955, revealed the visual acuity to be 20/20 in each eye with correction. The external, slit-lamp, and ophthalmoscopic examinations were within normal limits. There was an absolute, congruous left homonymous hemianopsia with sparing of the macula on visual field examination.

He was admitted to Emergency Hospital, where the general physical examination revealed a somewhat restless, well-developed white boy of 12 years of age. The examination was negative except for hyperesthesia over the entire left side, as compared with the right.

The laboratory findings were normal except for the spinal fluid, which showed 51 W. B. C. per cubic millimeter, with 96% lymphocytes and 4% neutrophiles. Spinal fluid studies showed 47% protein (normal 0 to 45%); 60 mg. of sugar per 150 cc., and 760 mg. of chlorides per 100 cc. Agglutination tests for lymphocytic choriomeningitis and eastern and western equine encephalitis viruses were negative; the toxoplasmosis dye test was negative.

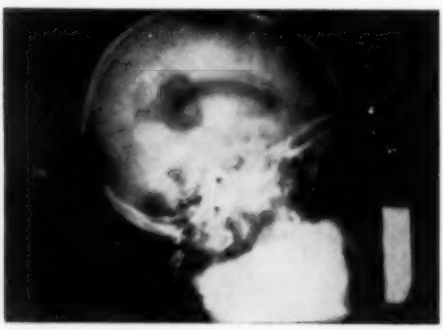
On Feb. 7, pneumoencephalography was performed, and the manometric pressure readings were normal. All ventricles were slightly dilated and were in the midline. There was a large porencephalic cyst, approximately 8 cm. in diameter, lying in the right posterior parietal area and communicating with the posterior portion of the body of the right lateral ventricle. The cyst depressed

Fig. 5.—Anteroposterior pneumoencephalogram, showing a large porencephalic cyst in the right posterior parietal area communicating with the posterior portion of the right lateral ventricle. All ventricles were slightly dilated. The occipital and temporal segments of the right lateral ventricle are depressed.

the occipital and temporal segments of the right lateral ventricle.

The patient was discharged from the hospital on Feb. 10, for follow-up care in the office. Examination on Feb. 26 showed that the headaches had subsided and there was recovery of the visual field, so that a left lower homonymous quadrantanopia was present.

Fig. 6.—Lateral pneumoencephalogram, showing a porencephalic cyst in the right posterior parietal region.



On April 2 the visual field was found to have completely recovered. The patient was symptom-free and has remained so at the date of the present writing.

COMMENT

The course of events in this patient's medical history is interesting. The causes for the cyst are, of course, obscure, but possible ones may be (1) trauma to the right temporal region at 2 years of age and (2) high fever with convulsions at 3 years of age.

Fig. 7.—Lateral pneumoencephalogram with occiput inferior, showing a fluid level in the porencephalic cyst.

The diagnosis was made by the pneumoencephalogram, although, as Murphy and Garvin [12] have pointed out, the electroencephalogram was abnormal over the area involved. One can only speculate why visual hallucinations and homonymous hemianopsia developed. It is apparent that an intracranial inflammation was present, although a specific diagnosis was not made. It is conceivable that the cyst wall was adjacent to the optic radiations. By a combination of inflammation of the wall and increased pressure within the cyst, impingement upon the radiations

could have occurred in this position sufficient to impair vision in the distribution shown by the visual fields. Similarly, pressure upon the temporal lobe could have accounted for the visual hallucinations. Recovery of the field and resolution of the hallucinations and other symptoms probably occurred as the inflammation subsided.

Because operative intervention for this condition is contraindicated as long as the cyst communicates freely with the ventricular system and subarachnoid space, the treatment is aimed at controlling the Jacksonian convulsions by antiepileptic drugs. It is difficult to predict the future course of the disease in the patient presented, so that the prognosis must remain guarded.

SUMMARY

1. A review of the subject of porencephaly is presented.

2. It is pointed out that a porencephalic cyst must be considered in the differential diagnosis of any intracranial mass lesion and can be diagnosed preoperatively, during life, only by pneumoencephalography or ventriculography.

3. An interesting case of porencephalic cyst is reported.

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Glaucoma Surgery

An Evaluation of Cycloelectrolysis and Cyclodiathermy

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This lecture is given in memory of and to honor the great leader in ophthalmology, George Edmund de Schweinitz, affectionately called King George by his devoted students. As one of this group of admiring pupils who derived so much stimulation from him and his inspiring teaching, the personality trait which impressed me most was his great willingness to give freely of his precious time to advise and otherwise aid younger men. The young ophthalmologists who organized the Association for Research in Ophthalmology owe much to the wise counsel of de Schweinitz. His support during the second meeting of the Association, when he, Jackson, Wilmer, Lancaster, and Parker presided by turns at the meeting, did much to insure and inspire the continuation of the project.

The history of his medical career, which was climaxed by his election to the presidency of the American Medical Association in 1922, is well known and has been described elsewhere.[1] Because of his literary efforts, and researches and the influence of his scholarly attainments, when the history of ophthalmology in the 20th century is written, de Schweinitz will take his place with other great men, of the caliber of Jackson, Fuchs, Parsons, Morax, Duke-Elder, Nordenson, van der Hoeve, and Wilmer. The legend of his forceful personality, indefatigable work, and outstanding character will continue to inspire ophthalmologists to greater accomplishments. This lecture is one evidence of his continued influence, and the invitation to address you was accepted with a sense of deep appreciation and humility.

The subject, "Evaluation of Certain Operations for Glaucoma," has been chosen because of de Schweinitz' interest in this problem, the extreme difficulty in obtaining scientific information from the literature, and its great importance in preventing some of the 12% of blindness[2] caused by glaucoma.

The statistical survey of the results of certain operations for glaucoma * which will be presented was made in an effort to evaluate the long-range efficacy of these procedures. Only complete case histories of patients who were observed postoperatively for a minimum period of one year are included in the analysis, which stresses the following points: (1) the control of tension within normal limits without the use of miotics; (2) satisfactory control of tension with miotics; (3) the increase or preservation of the preoperative visual fields post-

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* The compilation of many of these data was made possible through the courtesy of the staffs of the New York Eye and Ear Infirmary; the Manhattan Eye and Ear Hospital; the Institute of Ophthalmology, Presbyterian Hospital, New York; the Wills Eye Hospital; the Department of Ophthalmology of the University of Pennsylvania, and Dr. F. H. Adler, Dr. H. Scheie, and Dr. Edward Hartmann, who permitted me to include the records of their private patients with my own records.

operatively †; (4) the increase or preservation of the preoperative visual acuity postoperatively; (5) the condition of the glaucomatous eye from the standpoint of whether the operative procedure was the primary operation; (6) the necessity for additional surgery to control tension, and (7) the evaluation of immediate and long-range postoperative complications.

ANTERIOR AND POSTERIOR CYCLOELECTROLYSIS AS COMPARED WITH ANTERIOR CYCLODIATHERMY AND RETROCILIARY DIATHERMY

The operations commonly employed to lessen the production of intraocular fluids will be considered because of their great importance in some otherwise hopeless situations and the more recent developments in this field of glaucoma surgery.

In performing operations which reduced the production of aqueous, the earlier pioneers, such as Shahan and Post,[3] Curran,[4] Verhoeff,[5] and Zimin,[6] had but few followers because of the fear of excessive destruction of the ciliary body with subsequent sympathetic ophthalmitis.

In 1933 Weve[7] performed superficial coagulations of the ciliary body (nonpenetrating cyclodiathermy). Four years later penetrating cyclodiathermy was suggested by Vogt.[8] Both these procedures, which are now referred to as anterior (penetrating or nonpenetrating) cyclodiathermy, were applied near the corneal limbus. Weekers and associates[9] made nonpenetrating diathermy applications at a distance of 7 to 8 mm. from the corneal limbus and used a pyrometer for determining temperature. This method, known as retrociliary diathermy, has been modified by Weekers,‡ who now employs a 0.5 mm. needle to make the diathermy applications to the same region, but through the conjunctiva, and has abandoned the pyrometer. He attempts to limit the applications so that the inflow of aqueous will not be reduced below 50%.

Schreck[10] coagulated the long posterior ciliary arteries with anodic electrolysis by passing an electrode, similar to a cyclodialysis spatula, under the sclera 8 to 10 mm. from the limbus in the suprachoroidal space, using 0.8 to 1.0 ma. of current for 10 seconds. He named this operation ciliocycloanolysis. Solid carbon dioxide snow was applied to the sclera by Bietti,[11] with some favorable results.

Galvanic current was first utilized by Schoeler[12] for retinal detachment, and study of the effect of cathode current on the retina § led me to apply this current to the ciliary body as a treatment for certain types of glaucoma.‖ Research work on rabbit eyes, the results of which were later applied to human eyes, revealed not only that galvanic current produced less destruction of the sclera, but also that shrinkage of the eyeball was negligible as compared with diathermy. The bident electrode was primarily employed by me § to shrink the globe in treating separation of the retina by means of diathermy applications to the sclera. The marked shrinking of the sclera produced by diathermy has been clearly demonstrated by Scheie[13] in his brilliant experiments. In applying galvanic current to the ciliary body, our greatest concern was that sympathetic ophthalmitis might develop, but so far this fear has been groundless. No proved case of sympathetic ophthalmitis has been observed following cyclodiathermy or cycloelectrolysis in eyes studied in this paper.

There have been some misconceptions concerning the difference between cycloelectrolysis and cyclodiathermy (penetrating and nonpenetrating). Each procedure produces atrophy of part of the ciliary body and ciliary nerves and reduces the formation of aqueous. In cycloelectrolysis, however, there is a more accurately controlled chemical decomposition of tissues, produced by the sodium hydroxide of the cathode pole from a galvanic current. In cyclodiathermy (penetrating and nonpene-

† Visual field data were inadequate in most hospital records.

‡ Weekers, L.: Personal communication to the author, September, 1954.

§ Berens, C., in discussion on Pischel.[13]

‖ References 14 to 16.

trating), a high-frequency current of more than 1,000,000 oscillations per second is employed. This current generates enough heat to cause instantaneous coagulation of the proteins present, skrinks and dehydrates surface tissue, and causes some sparking of tissue, with subsequent undesirable carbonization. Swan and Christensen [18] state that misapplied or excessive diathermy produces unnecessary injury to the tissues and destroys function by the heat induced. It is easy to demonstrate that the undesirable thinning, skrinking, and puckering of the sclera following cyclodiathermy is minimal after cycloelectrolysis and that the chemical dissolution of the tissues which is accomplished without heat may be more accurately controlled.

Weekers and Prijot,[19] in investigating the mode of action of retrociliary (posterior) diathermy by means of an electronic tonometric pressure test, conclude that the procedure diminished neither the resistance to aqueous flow nor the episcleral venous pressure (Table 1). The hypotensive effect of the operation results solely from a diminution of aqueous output, which is due to neurovascular changes produced by the action of the diathermy current on the ciliary body. Sheppard ¶ revealed that pathological findings in the epithelium of the ciliary body are almost identical when either anterior or posterior (retrociliary) diathermy is applied to the eyeball. In regard to the mode of action of nonperforating retrociliary diathermy, Weekers and Prijot [19] state that the mechanism of hypotension following retrociliary diathermy differs from that of other antiglaumatous operations. Retrociliary diathermy does not open new exit channels for the aqueous humor, nor does it lower the resistance in these channels; its hypotensive action is exclusively the result of a lasting reduction of the aqueous production (Table 2).

¶ Sheppard, L. B.: Personal communication, September, 1954.

TABLE 1.—*Effect of Nonperforating Retrociliary Diathermy on the Resistance to Aqueous Flow* *
(Fluorescein Test)

Type of Glaucoma	No. of Cases	Resistance, Mean	Tension (Mm. Hg), Mean
Chronic simple glaucoma			
Untreated	58	12.1	28.0
Diathermy	26	10.6	18.6
Aphakic glaucoma			
Untreated	13	17.7	48.0
Diathermy	4	17.0	22.0

* Weekers.[19]

TABLE 2.—*Coefficient of Flow (Fluorescein Instillation) in Untreated Chronic Glaucoma and After Nonpenetrating Retrociliary Diathermy* *

	No. of Cases	Coefficient of Flow, Mean	Tension (Mm. Hg), Mean
Untreated	20	0.43	28
Diathermy	5	0.29	18

* Weekers.[19]

Urrets-Zavalia [20] concludes that the most likely mechanism of anterior cyclodiathermy in the lowering of intraocular tension is the reduction in the secretory activity of the ciliary body, i. e., a reduction in the rate of transfer of the osmotically effective electrolytes into the aqueous.

The indications and contraindications for anterior or posterior cycloelectrolysis, cyclodiathermy, and retrociliary diathermy (penetrating or nonpenetrating) are identical. However, the tabulation of the results of these procedures, as shown in Table 9, demonstrates a greater percentage of control of tension following cycloelectrolysis than following cyclodiathermy.

INDICATIONS FOR ANTERIOR AND POSTERIOR CYCLOELECTROLYSIS, ANTERIOR CYCLODIATHERMY, AND RETROCILIARY DIATHERMY

1. *Chronic Simple (Wide-Angle) or Closed-Angle Glaucoma* (Table 3). — In chronic simple glaucoma, which in much recent literature is named open-angle glaucoma, the outflow tests have revealed that the main difficulty is diminished outflow.[21] Therefore, in chronic simple glaucoma a filtering operation is certainly indicated as a primary procedure, with procedures which diminish the inflow of aqueous reserved as

secondary operations when the first fails to control tension, or for those cases in which a filtering operation is contraindicated.

Cycloelectrolysis and other procedures to decrease inflow are certainly not the operations of choice in closed-angle glaucoma, because in the early stages peripheral iridotomy,[22] peripheral iridectomy,[23] or iridectomy [24] may be successful in controlling tension for years. In these cases in which anterior peripheral synechiae are found, or outflow tests # or monocular or binocular bulbar pressure tests [27] show that outflow is interfered with, a filtering operation should be performed. I usually perform iridocorneosclerectomy [28] unless the base pressure [29] is low and peak pressure is under 45 mm. Hg; then cyclodialysis has proved satisfactory. Therefore, in closed-angle glaucoma it is my firm belief that cycloelectrolysis, although it produces better results than cyclodiathermy, is contraindicated as a primary procedure unless operation on the iris or a filtering operation is contraindicated.

Most operations designed to reduce the flow of aqueous, and even cataract extractions, as has been shown by Weekers [30] and others, result in reduction of inflow for a limited time only. If the inflow of aqueous is reduced too drastically, the nutrition of the lens and cornea may suffer and hypotony may be a serious threat to the health of the eye.

2. *Acute Glaucoma* (Table 4).—The present study indicates that cycloelectrolysis controlled tension in a larger percentage of the eyes affected with acute glaucoma than anterior cyclodiathermy and/or retrociliary diathermy. I am convinced that some form of iridectomy is the operation of choice. However, if tension is not controlled by medical treatment and outflow is seriously impaired, iridocorneosclerectomy is preferred.

3. *Glaucoma in Aphakia* (Table 5).—Both cycloelectrolysis and anterior cyclodiathermy have produced fair results in controlling tension in aphakic eyes. However, these procedures should be reserved for cases in which cyclodialysis, iridectomy, and iridosclerectomy are contraindicated, or as a secondary procedure when other operations fail to normalize tension.

4. *Glaucoma Secondary to Uveitis* (Table 6).—In eyes in which tension is not controlled by medical treatment, including acetazoleamide (Diamox)[31] or retrobulbar injections of procaine and epinephrine or weak solutions of ethyl alcohol, and in eyes in which iridectomy, iridocorneosclerectomy, or other filtering operations are contraindicated (i. e., in cases of newly formed vessels in the anterior chamber or extensive atrophy of the conjunctiva), anterior cycloelectrolysis has been helpful, at least temporarily. However, the number of cases reported in this study is too small from which to draw valid statistical conclusions concerning the results of cycloelectrolysis and cyclodiathermy.

5. *Buphthalmos and Hydrophthalmos.*—As hypertension in hydrophthalmos is caused by obstruction of outflow, goniotomy [32] or goniopuncture [33] seems to be the operation of choice, except in certain complicated cases.

6. *Secondary Glaucoma — Miscellaneous Causes* (Table 7).—Because of the small number of eyes operated upon, the following conditions are evaluated as a group under secondary glaucoma—miscellaneous causes: (*a*) thrombosis of the central vein (results are usually discouraging); (*b*) rubeosis of the iris; (*c*) hemorrhagic glaucoma; (*d*) nevus flammeus; (*e*) secondary glaucoma resulting from trauma, and (*f*) sarcoidosis.

In my experience, no operative procedure has given consistently good results in rubeosis iridis, hemorrhagic glaucoma, or sarcoidosis, although cycloelectrolysis has apparently been more successful than cyclodiathermy in controlling tension. In traumatic secondary glaucoma it has been possible to control tension with iced compresses, phenylephrine (Neo-Synephrine), retrobulbar injections of procaine and epinephrine, with or without the use of ethyl alcohol, and repeated aspiration of the anterior chamber.[34] Before cycloelectrolysis is performed in cases of traumatic secondary

References 25 and 26.

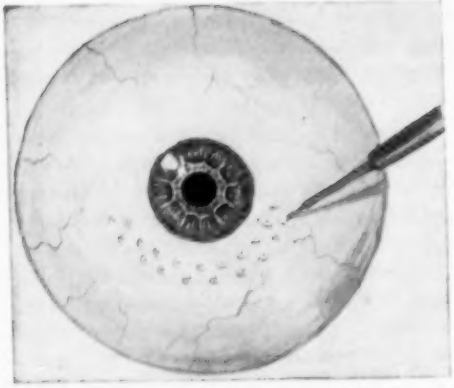


Fig. 1.—Anterior cycloelectrolysis. The galvanic punctures are applied through the conjunctiva between 2 and 4.5 mm. from the limbus, employing a fine conical straight needle 2 mm. long and 5 ma. of galvanic (cathode) current for five seconds.

glaucoma, the use of acetazoleamide should be considered.

7. *Aniridia with Glaucoma.*—In two eyes tension was controlled with miotics after cycloelectrolysis. My experience, though limited, has led me to prefer cyclodialysis as a primary procedure in these cases, although Barkan [32] has had success with goniotomy.

8. *Absolute Glaucoma* (Table 8).—In the management of absolute glaucoma, anterior cycloelectrolysis has proved efficacious in certain cases.

CONTRAINDICATIONS FOR CYCLOELECTROLYSIS AND CYCLODIATHERMY

Absolute contraindications for cycloelectrolysis and cyclodiathermy are few, but include intraocular tumors, glaucoma capsulare, and dislocation of the lens into the anterior chamber.

APPARATUS REQUIRED FOR CYCLOELECTROLYSIS

A galvanic battery or apparatus with a potential of at least 22.5 volts and a milliampere scale large enough to show small variations between 0 and 10 ma. are required.

The positive, or dispersive, electrode is placed somewhere on the patient's skin, usually under one shoulder, using an electrolytic jelly to insure a good contact. The negative pole, or active electrode, is a specially designed platinum conical, straight electrolysis needle, measuring 2.0 mm. in length and 0.18 mm. in diameter.

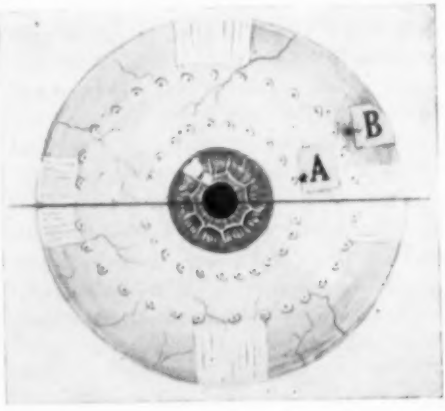
The setting of the rheostat is determined by applying the needle to the conjunctiva and adjusting the rheostat so that the milliammeter registers the current desired, usually 5 ma. for five seconds, although in some particularly sensitive patients it is desirable to use only 2 or 3 ma. of current and lengthen the duration of the applications for 8 to 10 seconds.

TECHNIQUE OF ANTERIOR OR POSTERIOR CYCLOELECTROLYSIS

Anterior cycloelectrolysis was the first technique employed, and most of the operations, both clinical and experimental, reported in this paper were performed with this technique. In anterior cycloelectrolysis, from 35 to 75 punctures are usually made in the inferior half of the eyeball, between 2 and 4.5 mm. from and parallel to the corneal limbus. A fine straight conical needle, 2.0 mm. in length, is inserted through the conjunctiva, and for each puncture 5 ma. of galvanic current (cathode) is applied for five seconds (Fig. 1).

A single or double row of punctures may be applied completely surrounding the cornea

Fig. 2.—*A*, anterior circumcorneal cycloelectrolysis. A single or double circumcorneal row of punctures may be applied between 2 and 4.5 mm. from the limbus, avoiding the horizontal meridian. *B*, posterior cycloelectrolysis. A row of punctures is applied 6 mm. from the limbus, using 5 ma. of galvanic current (cathode) for five seconds.



(anterior circumcorneal cycloelectrolysis), avoiding the horizontal meridian (Fig. 2*A*), or a complete ring of punctures is placed 6 mm. from the cornea (posterior circumcorneal cycloelectrolysis; Fig. 2*B*), avoiding the horizontal meridian.

In posterior intermuscular cycloelectrolysis a single or double row of electrolysis punctures is applied between the rectus muscles, 5 to 6 mm. from the corneal limbus (Fig. 3), using a fine needle (1.5 mm. in length). Sheppard's experiments in rabbit eyes have shown more reaction in the ciliary processes with this procedure than with the anterior cycloelectrolysis, and retrociliary diathermy has resulted in a large number of retinal separations. For this reason, I shall perform no more retrociliary electrolysis or diathermy until more carefully compiled statistics concerning the results of these procedures are available.

If tension remains high immediately following surgery, from 0.5 to 1.0 cc. of aqueous may be aspirated from the anterior chamber (Fig. 4).

POSTOPERATIVE TREATMENT FOLLOWING CYCLOELECTROLYSIS

A solution of atropine (1%) is instilled, and 1:3000 nitromersol (Metaphen) ointment and a monocular dressing are applied. The head and torso should be elevated, but

Fig. 3.—Posterior intermuscular cycloelectrolysis. A single or double row of electrolysis punctures is applied between the rectus muscles, 5 to 6 mm. from the corneal limbus.

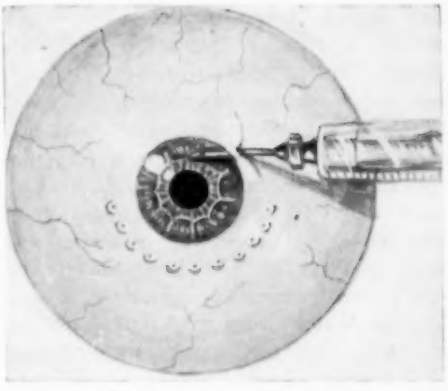


Fig. 4.—If the tension remains high immediately following surgery, from 0.5 to 1.0 cc. of aqueous may be aspirated from the anterior chamber.

rest in bed is unnecessary. The postoperative reaction is usually so slight that the operation, in some cases, may be performed as an office procedure. Scopolamine hydrobromide (0.25%) or additional instillations of atropine (1%) may be helpful in lessening congestion and in producing mild dilatation of the pupil.

Cortisone and iced compresses seem to lessen postoperative reaction. Sheppard [88] concludes that the clinical advantage gained by having an eye comfortable and with reduced inflammation after surgery by the use of cortisone is striking. However, cortisone may mask a serious infection or retard the normal processes of healing. It would seem advisable, therefore, not to use cortisone immediately after cycloelectrolysis and cyclodiathermy, or, if its use is necessary, to administer antibiotics at the same time.

PENETRATING AND NONPENETRATING ANTERIOR CYCLODIATHERMY AND RETROCILIARY DIATHERMY

Of a total of 264 eyes critically followed postoperatively for from 1 to 14 years, penetrating anterior cyclodiathermy was performed in approximately two-thirds, using a 1.5 mm. needle and applying the punctures from 2 to 4 mm. from the corneal limbus for five seconds for each puncture. Although the postoperative reactions in each type of cyclodiathermy were similar, it was noted

that slightly fewer postoperative complications followed nonpenetrating cyclodiathermy, but additional surgery to control tension was required in many more instances than following penetrating cyclodiathermy.

In a series of 127 eyes, retrociliary diathermy was performed as a primary procedure in 81%, and in the majority of this series the penetrating technique was employed. The punctures were made between the rectus muscles approximately 7 mm. from the limbus, using a 1.5 mm. needle for about five seconds. The postoperative results in this group, observed for a period of from one to four years, are more discouraging than those following anterior cyclodiathermy, in which the patients were followed for from one to 14 years (Tables 3 to 12).

Pathologic sections of rabbit eyes prepared one month after anterior penetrating or nonpenetrating cyclodiathermy showed necrosis and cellular infiltration into the episclera, and in some instances there was a rupture of the sclera on the treated side.

STATISTICAL EVALUATION OF ANTERIOR CYCLOELECTROLYSIS, ANTERIOR CYCLODIATHERMY, AND RETROCILIARY DIATHERMY

A comparative statistical evaluation of anterior cycloelectrolysis, anterior cyclodiathermy, and retrociliary diathermy is shown in Tables 3 through 12. Because only 11 eyes were treated by means of posterior cycloelectrolysis, and this number of operations is insufficient for valid statistical analysis, they are not included in these Tables. Of these 11 eyes, observed for from one to two years, the tension in 36% was controlled without miotics, and in 46%, with miotics; in 9% the tension was lowered between 30 and 40 mm. Hg, and in 9% the tension remained uncontrolled. Posterior cycloelectrolysis was used as a primary procedure in 54% of these cases, and additional surgery was required in 18% of the total number of eyes.

TABLE 3.—*Comparison of Postoperative Results in Chronic Simple Glaucoma Following Anterior Cyclodiathermy, Retrociliary Diathermy, and Anterior Cycloelectrolysis**

A. Operative procedure B. Primary operation C. Postoperative period of observation	No. of Eyes	Tension †					Enuc. or Evis.	Vision ‡			Additional Surgery
		1	2	3	4	5		1	2	3	
A. Anterior cyclodiathermy	100	5	32	22	21	8	12	6	4	78	37%
B. 82%	..	5%	32%	22%	21%	8%	12%	7%	5%	88%	
C. 1 to 14 yr.		37%						12%			
A. Retrociliary diathermy	55	1	19	17	12	4	2	13	12	28	26%
B. 68%	..	2%	34%	31%	22%	7%	4%	24%	23%	53%	
C. 1 to 4 yr.		36%						47%			
A. Anterior cycloelectrolysis	93	36	37	12	5	0	3	34	24	32	15%
B. 17%	..	20%	40%	13%	4%	..	4%	38%	26%	36%	
C. 2 to 9 yr.		79%						64%			

* The statistics included in Tables 3 through 12 were obtained through the courtesy of the staffs of the New York Eye and Ear Infirmary; the Manhattan Eye and Ear Hospital; the Institute of Ophthalmology, Presbyterian Hospital, New York; Wills Eye Hospital, Philadelphia, and the Department of Ophthalmology of the University of Pennsylvania. The records of Drs. Adler, Scheie, and Hartmann are included with my own records.

† Tension: 1 = controlled (under 25 mm. Hg, Schiøtz) without miotics; 2, controlled with miotics; 3, tension reduced (30-40 mm. Hg); 4, tension uncontrolled; 5, eye hypotonic.

‡ Vision: 1 = improved; 2, unchanged; 3, decreased or lost.

CHRONIC SIMPLE GLAUCOMA

Results obtained in chronic simple glaucoma by the use of anterior cyclodiathermy, retrociliary diathermy, and anterior cycloelectrolysis are shown in Table 3.

Anterior cyclodiathermy was employed as a primary procedure in 82% of a total of 100 eyes affected with chronic simple glaucoma, which were observed over a period of from 1 to 14 years. In only 5% of the eyes was tension controlled under 25 mm. Hg (Schiøtz) without miotics and in 32% with miotics, making a total of 37% of the eyes in which tension was satisfactorily controlled. Over one-third of the retained 88 eyes required additional surgery for control of tension. Retrociliary diathermy was used as a

primary procedure in 68% of 55 eyes observed for a period of from one to four years. In only 2% of the eyes was tension controlled under 25 mm. Hg without miotics and in 34% with miotics, making a total of 36% in which tension was controlled. Twenty-six per cent of the retained 53 eyes required additional surgery. Since the postoperative time factor is so important following all glaucoma surgery, the results of retrociliary diathermy are more discouraging than those following anterior cyclodiathermy when eyes have been observed over a longer period of time.

In spite of the fact that cycloelectrolysis was employed as a primary procedure in only 17% of the 93 eyes with chronic simple glaucoma (observed for from two to nine years postoperatively), the results are more encouraging than those following cyclodiathermy. However, the postoperative period of observation was shorter than that of the cyclodiathermy cases. Thirty-nine per cent were controlled without miotics and 40% with miotics, for a total of 79% in which tension was controlled under 25 mm. Hg. Fifteen per cent of the retained 90 eyes required additional surgery. Visual results were statistically better following anterior cycloelectrolysis than following anterior cyclodiathermy or retrociliary diathermy.

Acute Glaucoma

Results obtained in acute glaucoma by the use of anterior cyclodiathermy, retrociliary diathermy, and anterior cycloelectrolysis are shown in Table 4.

In this small series of eyes affected with acute glaucoma, although tension by anterior cyclodiathermy was controlled in none without miotics, the over-all results (22% controlled with miotics) are slightly better than those following retrociliary diathermy, showing only 20% of eyes in which tension was controlled with and without miotics. The eyes upon which retrociliary diathermy was performed were observed over a shorter period of time, but required additional surgery in more instances than following anterior cyclodiathermy. The postoperative results in the 15 eyes following cycloelectrolysis were better than those following anterior cyclodiathermy and retrociliary diathermy, for tension was controlled in 66% of the eyes, with and without miotics, and vision was improved or unchanged in a higher percentage of eyes.

Table 4.—*Comparison of Postoperative Results in Acute Glaucoma Following Anterior Cyclodiathermy, Retrociliary Diathermy, and Anterior Cycloelectrolysis*

A. Operative procedure B. Primary operation C. Postoperative period of observation	No. of Eyes	Tension *					Enuc. or Evis.	Vision †			Additional Surgery
		1	2	3	4	5		1	2	3	
A. Anterior cyclodiathermy	32	0	7	8	11	2	4	2	2	14	18%
B. 88%	..	..	22%	25%	34%	6%	13%	7%	7%	86%	
C. 1 to 14 yr.								14%			
A. Retrociliary diathermy	15	2	1	1	7	0	3	2	2	8	33%
B. 58%	..	13%	7%	13%	47%	..	20%	17%	17%	66%	
C. 1 to 4 yr.		20%						34%			
A. Anterior cycloelectrolysis	15	5	5	4	0	0	1	5	4	5	21%
B. 43%	..	33%	33%	27%	..	..	7%	36%	28%	36%	
C. 2 to 9 yr.		66%						64%			

* Tension: 1 = controlled (under 25 mm. Hg, Schiötz) without miotics; 2, controlled with miotics; 3, tension reduced (30-40 mm. Hg); 4, tension uncontrolled; 5, eye hypotonic.
† Vision: 1 = improved; 2, unchanged; 3, decreased or lost.

Aphakic Glaucoma

The postoperative results in aphakic glaucoma following anterior cycloelectrolysis, retrociliary diathermy, and anterior cyclodiathermy are shown in Table 5.

Tension was controlled in a total of 35% of 51 aphakic glaucomatous eyes following anterior cyclodiathermy, as compared with only 15% of 21 eyes following retrociliary diathermy observed over a much shorter postoperative period. One-fourth of the eyes in which retrociliary diathermy was per-

TABLE 5.—*Comparison of Postoperative Results in Aphakic Glaucoma Following Anterior Cyclodiathermy, Retrociliary Diathermy, and Anterior Cycloelectrolysis*

A. Operative procedure B. Primary operation C. Postoperative period of observation	No. of Eyes	Tension *					Enuc. or Evis.	Vision †			Additional Surgery
		1	2	3	4	5		1	2	3	
A. Anterior cyclodiathermy	51	4	14	11	11	6	5	3	2	41	22%
B. 55%	..	8%	27%	22%	22%	12%	9%	6%	4%	90%	
C. 1 to 14 yr.		35%						10%			
A. Retrociliary diathermy	21	1	2	7	8	2	1	2	3	15	25%
B. 70%	..	5%	10%	30%	30%	10%	5%	10%	15%	75%	
C. 1 to 4 yr.		15%						25%			
A. Anterior cycloelectrolysis	35	8	15	6	4	0	2	10	3	20	21%
B. 70%	..	23%	42%	17%	12%	..	6%	30%	9%	61%	
C. 2 to 9 yr.		65%						39%			

* Tension: 1 = controlled (under 25 mm. Hg, Schiøtz) without miotics; 2, controlled with miotics; 3, tension reduced (30-40 mm. Hg); 4, tension uncontrolled; 5, eye hypotonic.

† Vision: 1 = improved; 2, unchanged; 3, decreased or lost.

formed required additional surgery for control of tension in the retained eyes. The number of eyes with visual improvement following both these procedures is small (6% following anterior cyclodiathermy and 10% following retrociliary diathermy).

The results in 35 eyes following anterior cycloelectrolysis were more encouraging, for 23% were controlled without miotics and 42% with miotics, making a total of 65% of the eyes in which tension remained below 25 mm. Hg. Twenty-one per cent of the retained 33 eyes required additional surgery, and visual improvement was noted in 30% of the eyes. It is important to consider that the postoperative period of observation of some of the eyes treated by diathermy was longer than that in eyes in which electrolysis was applied.

GLAUCOMA SECONDARY TO UVEITIS

The postoperative results of anterior cyclodiathermy and anterior cycloelectrolysis in glaucoma secondary to uveitis are tabulated in Table 6.

The postoperative results in this limited number of eyes affected with glaucoma secondary to uveitis are startling, and definitely indicate that cyclodiathermy is not the procedure of choice in this condition. Only one-fifth of the 10 eyes treated by means of anterior cyclodiathermy were controlled with miotics, and an additional one-fifth of the eyes in this series were enucleated. Twelve per cent of the retained eyes required additional surgery, and only one eye showed visual improvement. Of 21 eyes following cycloelectrolysis, tension was controlled in 29% without miotics and in 33% with miotics, making a total of 62% with tension controlled under 25 mm. Hg (Schiøtz). One-half these eyes had had previous operations, and 10% of the retained 20 eyes required additional surgery. Visual improvement was recorded in 50% of the retained eyes.

TABLE 6.—*Comparison of Postoperative Results of Anterior Cyclodiathermy and Anterior Cycloelectrolysis in Glaucoma Secondary to Uveitis*

A. Operative procedure B. Primary operation C. Postoperative period of observation	No. of Eyes	Tension *					Enuc. or Evis.	Vision †			Additional Surgery
		1	2	3	4	5		1	2	3	
A. Anterior cyclodiathermy	10	0	2	2	4	0	2	1	0	7	12%
B. 62%	..	..	20%	20%	40%	..	20%	12%	..	88%	
C. 1 to 14 yr.											
A. Anterior cycloelectrolysis	21	6	7	5	2	0	1	10	5	5	10%
B. 50%	..	29%	33%	24%	9.5%	..	4.5%	50%	25%	25%	
C. 2 to 8 yr.		62%						75%			

* Tension: 1 = controlled (under 25 mm. Hg, Schiøtz) without miotics; 2, controlled with miotics; 3, tension reduced (30-40 mm. Hg); 4, tension uncontrolled; 5, eye hypotonic.

† Vision: 1 = improved; 2, unchanged; 3, decreased or lost.

Buphthalmos and Hydrophalmos

Fifteen cases of buphthalmos were treated by means of anterior cycloelectrolysis, which was the primary procedure in 25% of the eyes. These patients were observed over a period of from three to eight years. Tension was controlled under 25 mm. Hg without miotics in 33% of the eyes and with miotics in 27%, making a total of 60% of the eyes in which tension was controlled. In an additional 27% the tension was lowered (between 30 and 40 mm. Hg), but in the remaining 13% of the eyes tension was uncontrolled. Additional surgery was performed in 12.5% of the eyes. Vision was improved in 46% of the eyes, remained unchanged in 27%, and was reduced or lost in 27%. In our series there were no cases of buphthalmos treated by means of anterior cyclodiathermy or retrociliary diathermy. Allbough and Dunphy [36] reported that of four eyes treated with nonpenetrating cyclodiathermy for buphthalmos, two obtained successful results and two were failures. Scheie [37] described two cases of buphthalmos in which goniotomy had failed to lower tension but cyclodiathermy subsequently normalized tension.

Secondary Glaucoma—Miscellaneous Causes

An insufficient number of cases of secondary glaucoma due to thrombosis of the central vein, rubeosis of the iris, trauma, sarcoidosis, or hemorrhagic glaucoma, as well as nevus flammeus associated with glaucoma, have been observed after cyclodiathermy and cycloelectrolysis to permit a valid statistical evaluation for each condition. These cases, therefore, are grouped together and tabulated as secondary glaucoma—miscellaneous causes (Table 7). The results seem to indicate that cycloelectrolysis produced better results than cyclodiathermy.

Absolute Glaucoma

The postoperative results in 25 eyes with absolute glaucoma following anterior cycloelectrolysis were superior to those following anterior cyclodiathermy in the same number of eyes. However, in comparing the results in Table 8, one must consider that the period of observation of the cycloelectrolysis eyes was shorter, and that the operation was performed as a primary procedure in 90% of the eyes, as compared with 77% in which cyclodiathermy was used. In none of the three eyes treated with retrociliary diathermy was tension controlled, in spite of the fact that additional surgery was performed in all three eyes.

Compilation of Results in All Types of Glaucoma

The postoperative results following anterior cyclodiathermy, retrociliary diathermy, and anterior cycloelectrolysis for all types of glaucoma are shown in Table 9.

From the evaluation of the postoperative results tabulated in Table 9, cycloelectrolysis seems superior to cyclodiathermy when all types of glaucoma are combined, in spite of the fact that the procedure was used as a primary operation in only 35% of the 239 cases. However, in making a final evaluation of the results, one must take into consideration the fact that some of the anterior cyclodiathermy eyes were observed for from 1 to 14 years, as compared with 2 to 9 years for anterior cycloelectrolysis eyes. Only 19% of the patients treated with cycloelectrolysis required additional surgery for control of tension, which was normal in 32% of the cases without miotics and in 33% with miotics, making a total of 65% in which tension remained under 25 mm. Hg.

Anterior cyclodiathermy was employed as a primary procedure in 70% of the 264 eyes, and additional surgery was required in 31% in order to control tension in 4% of the eyes without miotics and in 24% with miotics, making a total of 28% of the eyes in which tension was controlled.

Evaluation of Anterior Cyclodiathermy and Anterior Cycloelectrolysis in All Types of Glaucoma in Relation to the Postoperative Period of Observation

Anterior Cyclodiathermy.—In following the case histories of patients in whom cyclo-

TABLE 7.—*Comparison of Postoperative Results in Secondary Glaucoma—Miscellaneous Causes—Following Anterior Cyclodiathermy, Retrociliary Diathermy, and Anterior Cycloelectrolysis*

A. Operative procedure B. Primary operation C. Postoperative period of observation	No. of Eyes	Tension *					Enuc. or Evis.	Vision †			Additional Surgery
		1	2	3	4	5		1	2	3	
A. Anterior cyclodiathermy	46	2	4	8	19	4	9	2	2	33	33%
B. 70%	..	4%	9%	18%	41%	9%	19%	6%	6%	88%	
C. 1 to 14 yr.		13%						12%			
A. Retrociliary diathermy	33	0	6	2	13	6	6	1	3	23	39%
B. 93%	..	..	18%	6%	39%	18%	18%	3%	11%	86%	
C. 1 to 4 yr.								14%			
A. Anterior cycloelectrolysis	35	11	4	11	5	1	3	6	4	22	35%
B. 32%	..	31%	12%	31%	15%	3%	8%	19%	13%	68%	
C. 2 to 9 yr.		43%						32%			

* Tension: 1 = controlled (under 25 mm. Hg, Schiøtz) without mioties; 2, tension with mioties; 3, tension reduced (30-40 mm. Hg); 4, tension uncontrolled; 5, eye hypotonic.
† Vision: 1 = improved; 2, unchanged; 3, decreased or lost.

TABLE 8.—*Comparison of Postoperative Results in Absolute Glaucoma Following Anterior Cyclodiathermy, Retrociliary Diathermy, and Anterior Cycloelectrolysis*

A. Operative procedure B. Primary operation C. Postoperative period of observation	No. of Eyes	Tension*					Enuc. or Evis.	Additional Surgery
		1	2	3	4	5		
A. Anterior cyclodiathermy	25	0	4	5	6	1	9	44%
B. 77%	..	..	16%	20%	24%	4%	36%	
C. 1 to 14 yr.								
A. Retrociliary diathermy	3	0	0	1	1	0	1	3 eyes
B. 3 eyes								
C. 1 to 4 yr.								
A. Anterior cycloelectrolysis	25	5	7	6	3	0	4	29%
B. 96%	..	20%	28%	24%	12%	..	16%	
C. 2 to 9 yr.		48%						

* Tension: 1 = controlled (under 25 mm. Hg, Schiøtz) without mioties; 2, tension with mioties; 3, tension reduced (30-40 mm. Hg); 4, tension uncontrolled; 5, eye hypotonic.

TABLE 9.—*Comparison of Postoperative Results in all Types of Glaucoma Following Anterior Cyclodiathermy, Retrociliary Diathermy, and Anterior Cycloelectrolysis*

A. Operative procedure B. Primary operation C. Postoperative period of observation	No. of Eyes	Tension *					Enuc. or Evis.	Vision †			Additional Surgery
		1	2	3	4	5		1	2	3	
A. Anterior cyclodiathermy	264	11	63	56	72	21	41	14	26	183	31%
B. 70%	....	4%	24%	21%	27%	8%	16%	6%	11%	83%	
C. 1 to 14 yr.		28%						17%			
A. Retrociliary diathermy	127	4	28	29	41	12	13	18	22	74	30%
B. 81%	....	4%	22%	23%	32%	9%	10%	16%	19%	65%	
C. 1 to 4 yr.		26%						35%			
A. Anterior cycloelectrolysis	239	77	79	47	21	1	14	72	65	88	19%
B. 35%	....	32%	33%	20%	9%	..	6%	33%	29%	38%	
C. 2 to 9 yr.		65%						62%			

* Tension: 1 = controlled (under 25 mm. Hg, Schiøtz) without mioties; 2, controlled with mioties; 3, tension reduced (30-40 mm. Hg); 4, tension uncontrolled; 5, eye hypotonic.
† Vision: 1 = improved; 2, unchanged; 3, decreased or lost.

diathermy or cycloelectrolysis was performed, it is important to note that when cyclodiathermy is performed tension is controlled without mioties in fewer eyes, a larger number of degenerations of the eyeball occur, and additional surgery is required in more instances in order to control the tension. This is particularly evident in an isolated series of 67 eyes which were observed over a period of from 10 to 14 years following anterior cyclodiathermy. In none of these eyes was tension controlled without mioties, and, although additional surgery was performed in 35% of the cases, tension was

controlled with miotics in only 14% of the eyes. Nine per cent of the retained eyes were hypotonic, and an additional 20% were enucleated because of serious complications. In Table 10 the results following anterior cyclodiathermy in patients observed for from 7 to 14 years and those followed postoperatively for from 1 to 6 years are compared.

It may be seen from Table 10 that in only 22% of the eyes observed for from 7 to 14 years in which anterior cyclodiathermy was used was tension controlled under 25 mm. Hg, as compared with 35% in the group observed for from 1 to 6 years. Furthermore, enucleation or evisceration was performed in 19% of the first group and in 11% of the second group. Visual acuity remained the same or was improved in only 12% of the group observed longer postoperatively, and additional surgery was required in 30% of these eyes. In the one- to six-year group 25% of the eyes were recorded as having the same or improved vision, but only 33% required additional surgery in order to control tension.

Anterior Cycloelectrolysis—Tension seems to have been controlled better and deterioration of the eye and visual acuity did not seem so rapid following cycloelectrolysis, as compared with the results of cyclodiathermy performed for all types of glaucoma, as revealed in Table 11.

The compilation of 239 eyes affected with all types of glaucoma in which cycloelectrolysis was performed reveals that in Group A, observed for from seven to nine years, there were fewer eyes (20%) in which tension was controlled without miotics than in Group B, in which the eyes were observed for from two to six years and in which tension was controlled under 25 mm. Hg in 35% without miotics. The gradual lessening of effectiveness in the control of tension is true of all operations for glaucoma and is also noticeable following cycloelectrolysis and cyclodiathermy, as clearly demonstrated

TABLE 10.—*Comparison of Results, Including the Postoperative Time Factor, Following Anterior Cyclodiathermy for all Types of Glaucoma*

Postoperative Period of Observation	Primary Surgery Mean	No. of Eyes	Tension* 1	2	3	4	5	Enuc. or Evis.	Vision† 1	2	3	Additional Surgery
Group A (7 to 14 yr.)	72%	150	3	33	34	42	12	28	5	10	107	30%
	....	...	1%	21%	22%	29%	8%	19%	4%	8%	88%	
			22%						12%			
Group B (1 to 6 yr.)	71%	114	9	31	22	30	9	13	9	16	76	33%
	....	...	8%	27%	20%	26%	8%	11%	9%	16%	75%	
			35%						25%			

* Tension: 1 = controlled (under 25 mm. Hg, Schiøtz) without miotics; 2, controlled with miotics; 3, tension reduced (30-40 mm. Hg); 4, tension uncontrolled; 5, eye hypotonic.
† Vision: 1 = improved; 2, unchanged; 3, decreased or lost.

TABLE 11.—*Comparison and Results, Including Postoperative Time Factor, Following Anterior Cycloelectrolysis in All Types of Glaucoma*

Postoperative Period of Observation	Primary Surgery Mean	No. of Eyes	Tension* 1	2	3	4	5	Enuc. or Evis.	Vision† 1	2	3	Additional Surgery
Group A (7 to 9 yr.)	39%	40	8	16	9	3	0	4	8	9	19	25%
	....	..	20%	40%	22%	8%	..	10%	22%	25%	53%	
			60%						47%			
Group B (2 to 6 yr.)	40%	199	69	63	38	18	1	10	64	56	69	14%
	....	..	35%	32%	18%	9%	..	5%	34%	29%	37%	
			67%						63%			

* Tension: 1 = controlled (under 25 mm. Hg, Schiøtz) without miotics; 2, controlled with miotics; 3, tension reduced (30-40 mm. Hg); 4, tension uncontrolled; 5, eye hypotonic.
† Vision: 1 = improved; 2, unchanged; 3, decreased or lost.

in Tables 10 and 11. In Group A, 10% of the eyes were enucleated or eviscerated, as compared with 5% in Group B. Visual acuity was the same or improved in 47% of eyes in Group A and in 63% in Group B.

POSTOPERATIVE COMPLICATIONS FOLLOWING ANTERIOR CYCLODIATHERMY, RETROCILIARY DIATHERMY, AND ANTERIOR CYCLOELECTROLYSIS

The postoperative complications following anterior cyclodiathermy, retrociliary diathermy, and anterior cycloelectrolysis in retained eyes, observed for from 1 to 14 years, are shown in Table 12.

Table 12.—*Postoperative Complications in Retained Eyes Following Anterior Cyclodiathermy, Retrociliary Diathermy and Anterior Cycloelectrolysis*

Complications	Anterior Cyclodiathermy (223 Eyes Observed from 1 to 14 yr.)		Retrociliary Diathermy (114 Eyes Observed from 1 to 4 yr.)		Anterior Cycloelectrolysis (220 Eyes Observed from 2 to 9 yr.)	
	No.	%	No.	%	No.	%
Atrophic globe	24	11	8	6.5	2	1
Phthisis bulbi	9	4	2	2	1	..
Hemorrhage, anterior chamber	19	9	6	5	0	..
Vitreous hemorrhage	7	3	1	1	0	..
Uveitis	9	4	0	..	0	..
Cataract	5	2	0	..	0	..
Retinal detachment	2	1	0	..	1	..
Acute iritis	3	1+	0	..	1	..
Iridocyclitis	8	3	1	1	3	1+
Absolute glaucoma	11	6	1	1	6	3
Bullous keratitis	2	1	0	..	0	..
Panophthalmitis	0	..	2	2	0	..

The large percentage of serious complications following anterior and posterior cyclodiathermy seems to indicate clearly that thermal destruction of the ciliary body should be carefully evaluated by those who want to employ this method for reducing the formation of aqueous. Several ophthalmologists (Swan,* Argañaraz,† and Hartmann ‡) have completely abandoned cyclodiathermy because of the poor results and the serious complications encountered. Hartmann ‡ stated that cycloelectrolysis has completely replaced cyclodiathermy in his clinic for the past three years. He submitted the postoperative results obtained in 53 eyes treated with cycloelectrolysis (these have been included in Tables 3 to 11). There were 26 cases of primary glaucoma, 15 cases of secondary hypertensive glaucoma, 5 cases of aphakic glaucoma, and 7 cases of buphthalmos. Fifty-nine per cent of these eyes had had previous surgery, and cycloelectrolysis was repeated in three eyes. Of the 53 eyes, tension was controlled in 49% without miotics and 17% with miotics, making a total of 66% of eyes in which tension was controlled under 25 mm. Hg. Hartmann ‡ stated that the patients upon whom he performed cycloelectrolysis were poor surgical risks, and he therefore believes that excellent results were obtained in this group.

As seen in Table 12, the incidence of phthisis bulbi and atrophy of the globe (15%) appears relatively high following anterior cyclodiathermy. However, a questionnaire sent to members of the American Academy of Ophthalmology and Otolaryngology and to ophthalmologists abroad revealed that 19% of 1078 eyes became atrophic following cyclodiathermy, and phthisis bulbi occurred in an additional 9% of these retained eyes, making a total of 28% of eyes so affected. Lachman and Rockwell [38] reported poor results in a series of 39 eyes treated with retrociliary cyclodiathermy, as recommended by Castroviejo. Atrophy of the globe developed in 15% of the eyes. Accord-

* Swan, K.: Personal communication to the author, 1949.

† Argañaraz, R.: Personal communication to the author, 1949.

‡ Hartmann, E.: Personal communication to the author, 1954.

ing to de Roetth,[39] the high incidence of atrophy of the eyeball following cyclodiathermy (20% of 40 eyes operated on) is sufficient to discredit the operation.

No proved case of sympathetic ophthalmitis has been observed following cyclodiathermy and cycloelectrolysis in the eyes studied in this paper. Bodian[40] reported a case of sympathetic ophthalmitis which developed three and one-half months after surface and partially penetrating cyclodiathermy. He concludes that if sympathetic ophthalmitis occurs after nonperforating cyclodiathermy alone, the procedure should be contraindicated for blind eyes in the presence of a functioning fellow eye. Sympathetic ophthalmitis following cyclodiathermy has been reported by Bietti[41] (two cases), Streiff § (one case), and Thomas.[41] Woods ‖ reported one case of sympathetic ophthalmitis which developed after cyclodiathermy but stated that this probably resulted from injury which the eye has sustained, rather than from the application of diathermy.

GENERAL CONSIDERATIONS OF CYCLOELECTROLYSIS

If a surgeon wishes to lessen the formation of aqueous, cycloelectrolysis should be considered if other operations fail, or possibly as a primary procedure in selected cases when, for example, a filtering operation is contraindicated. If tension is not adequately controlled, the procedure may be repeated, without too great danger, in other quadrants of the eyeball after two weeks, or in the area where the electrolysis punctures were applied after one month.

SUMMARY

Postoperative results following certain surgical procedures employed for the control of glaucoma have been evaluated from the clinical records of five hospitals, and from the records of the private patients of Dr. F. H. Adler, Dr. Harold Scheie, Dr. Edward Hartmann, and myself. The primary purpose of the study was to evaluate certain operations over a long period of time to determine in what percentage of the eyes the procedures were employed as a primary operation; the number of eyes in which the various types of glaucoma were controlled under 25 mm. Hg, with and without miotics; the visual results; the complications encountered, and the number of patients who required additional surgery.

It has been found that most operations produced excellent results in patients followed postoperatively for relatively short periods of time, e.g., from one to two years, but that in those observed for several years additional operations were required to control tension in a larger number of eyes. The decrease in the percentage of eyes in which tension was controlled over a reasonably long postoperative period was found to be particularly striking following cyclodiathermy. Of 72% of 150 eyes in which cyclodiathermy was used as a primary procedure and the postoperative observation period was 7 to 14 years (Table 10), tension was controlled in only 1% without miotics and 21% with miotics, making a total of 22% in which tension was controlled under 25 mm. Hg. Of 114 eyes in which cyclodiathermy was employed as a primary procedure, 71% were observed for from one to six years postoperatively. In 8% of these eyes tension was controlled without miotics and 27% with miotics, making a total of 35% in which tension was controlled under 25 mm. Hg. Vision in 223 retained eyes following anterior cyclodiathermy was improved in 6% and unchanged in 11%, making a total of 17% in which preoperative vision was retained or improved after operation.

Postoperative complications following anterior cyclodiathermy in 223 retained eyes observed for from 1 to 14 years were as follows: atrophic globes (11%), phthisis bulbi (4%), hemorrhage into the anterior chamber (9%), vitreous hemorrhage (3%), uveitis (4%), cataract (2%), retinal de-

§ Streiff, E. B., in discussion on Wagner, H.: Bisherige Erfahrungen mit der Diathermistichelung des corpusciliare bei Glaukom, Schweizerische ophthalmologischen Gesellschaft, XXXI Jahresversammlung, Lucerne, June 18-19, 1938.

‖ Woods, A.: Personal communication to the author, 1949.

tachment (1%), acute iritis (1+%), iridocyclitis (3%), absolute glaucoma (6+%), and bullous keratitis (1%). Of the entire series of 264 eyes, 16% were enucleated.

The results of retrociliary diathermy in 127 eyes, observed for from one to four years, are most discouraging. Used as a primary procedure in 81% of the eyes, tension was controlled under 25 mm. Hg in only 4% of the eyes without miotics and in 22% with miotics, making a total of 26% in which tension was controlled. Thirty per cent of these eyes required additional surgery, and 10% of the eyes were enucleated. Vision in the retained 114 eyes was improved in 16% and remained unchanged in 10%. Of the retained 114 eyes, 6.5% became atrophic; phthisis bulbi occurred in 2%; hemorrhage into the anterior chamber, in 5.5%; vitreous hemorrhage, in 1%; iridocyclitis, in 1%, and panophthalmitis in 2%.

When these findings are compared with the results for 239 eyes observed for from two to nine years in which cycloelectrolysis was employed for all types of glaucoma, it seems that the chemical method (cycloelectrolysis) is more effective, with less serious complications, than the thermal (cyclodiathermy) method. Tension was controlled in 32% of the 239 eyes without miotics and in 33% with miotics, making a total of 65% in which tension was controlled under 25 mm. Hg. It is important to note that cycloelectrolysis was employed as a primary procedure in only 35% of these eyes, and additional surgery was required in 19% of this group (Table 9). Six per cent became atrophic; phthisis bulbi occurred in one case; retinal separation, in one case; iridocyclitis, in three cases, and absolute glaucoma, in 3% of the eyes.

Pathologic section of rabbit eyes treated by means of cyclodiathermy showed necrosis and cellular infiltration into the episclera. In some instances there was a rupture of the sclera on the treated side. The iris root and ciliary processes were engorged with blood, and the pigment epithelium was disturbed in some areas. The nonpigmented epithelium was absent in most sections. There were marked changes in the ciliary processes, but these were more marked following retrociliary diathermy.

An insufficient number of eyes were operated upon by means of posterior cycloelectrolysis to permit evaluation of this procedure.

Following anterior cycloelectrolysis, pathologic sections of rabbit eyes showed loss of the nonpigmented epithelium of the ciliary processes in certain areas and partial destruction of the pigmented epithelium.

CONCLUSION

Of the two most-used procedures designed to diminish the formation of aqueous, cycloelectrolysis seems to be more effective than cyclodiathermy, with fewer postoperative complications. The long-range prognosis following anterior cyclodiathermy is discouraging, and the results of retrociliary diathermy are poor, with a high percentage of atrophic eyes resulting from both these procedures. Because most increased tension results from diminished outflow, the hypotensive effect of operations like cyclodiathermy and cycloelectrolysis is often of short duration; these procedures should be reserved for cases in which filtering operations are contraindicated, or in which outflow following a filtering procedure is mildly inadequate.

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Cataracts Induced by Alloxan Diabetes in Rats and Rabbits

Experimental Studies

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The present study was undertaken with the object of determining whether the progression of cataract formation, when the latter is induced by diabetes, might be altered by the following procedures: (*a*) feeding of diets containing an excess of nicotinic acid, (*b*) the injection of cysteine, or (*c*) maintenance of the animals in an atmosphere of 38%-40% oxygen. In addition, a comparison has been made of cataract formation in rats and rabbits as observed with the ophthalmoscope. In order to verify and supplement these observations, the lenses have been studied histologically.

In a previous publication [1] it was noted that when diets containing excess nicotinic acid were fed to diabetic rats there was some delay in cataract formation. This could have resulted either from a lower food intake of the nicotinic-acid-fed group or from some effect of the nicotinic acid per se. In the present study the paired-feeding method was used in an effort to determine whether either of the above postulations is correct.

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* References 2 to 4.

Furthermore, von Sallmann * has shown that cysteine has some inhibitory effect on the development of radiation cataract. Also, Mitchell and Cook [5] found some retarding effect of cysteine on the development of galactose cataract. Thus, it was surmised that cysteine might also influence the development of diabetic cataracts, and so this material was used in one phase of the present study.

Moreover, it has been shown that the oxygen consumption is lower in the cataractous lens.[6] Consequently, an attempt has been made to delay the development of diabetic cataract by subjecting animals to an atmosphere containing 38%-40% oxygen.

METHODS

Adult male rats of the Long-Evans strain were fasted for 48 hours and given a subcutaneous injection of 115 mg. of alloxan per kilogram of body weight. The diabetic rats were followed, as in our previous study,[1] as to blood sugar, urine volumes, food intake, and body weight. For the nicotinic-acid phase of the study, the rats were paired as nearly as possible according to body weight and the severity of their diabetes. Of the animals that were paired, 13 were given a synthetic stock diet,[1] and 13 were given the stock diet plus 0.1% nicotinic acid. The animals given nicotinic acid were allowed to eat ad libitum, and their pair-mates were limited to the amount of food eaten each day by the former rats.

The same pair-feeding method was used in the cysteine study. For this phase of the work, half of the diabetic animals in the group were injected subcutaneously with 35 mg. of freshly neutralized cysteine twice daily.

In the third part of the study, animals were placed in a chamber in which air contained 38% to 40% of oxygen.† The animals were placed in this chamber immediately after the injection of alloxan. Since the rats were not in individual chambers, the food intake of each animal could

† Supplied through the courtesy of Linde Air Products, 230 N. Michigan Ave., Chicago.

not be compared with a control. The rate of cataract progression in these rats was compared with that in animals which had similar diabetic signs but were maintained in normal atmospheric conditions.

Albino rabbits weighing between 1.5 and 1.8 kg. were injected intravenously with 100 to 120 mg. of alloxan per kilogram of body weight. The optimum dose was 120 mg. per kilogram, since smaller doses did not make the animals permanently diabetic.

Both rats and rabbits were examined ophthalmoscopically at weekly intervals. The lens opacities in the animals were graded 1+ to 4+, as in our previous study,[1] and an attempt was made to localize the lesion in the lens.

For the histologic part of the study, the eyes were removed and opened just anterior to the equator. This opening was continued around the eyeball, and then, under direct visualization, the lens was removed from the anterior segment. This was facilitated by cutting the zonule; otherwise, especially in the rabbit, the capsule of the lens was broken.

The lenses were fixed in the Carnoy-LeBrunn fixative for 24 hours. After this, they were embedded in paraffin in the usual manner, but before sectioning the blocks of tissue were immersed in isotonic saline for 24 hours. The lenses were cut at 10μ and usually stained with hematoxylin and eosin.

I. OPHTHALMOSCOPIC OBSERVATIONS

A. Rats. — The first ophthalmoscopic change observed was either an anterior or a posterior subcapsular opacity, the former being seen more frequently. Occasionally equatorial vacuoles were the first evidence of cataract. When the latter were seen, they were usually followed within a week by anterior or posterior subcapsular opacities. These opacities enlarged, frequently appearing to follow the suture lines, and subsequently anterior or posterior cortical changes developed, or on occasions the two appeared simultaneously. These subcapsular and cortical changes increased and extended to the periphery of the lens, and eventually no fundus reflex could be seen. If the severely diabetic animal was allowed to survive, in most instances the lens became swollen and contained several water slits.

In general, it required a longer period of time for diabetic rats to develop lens opacities when their blood sugar levels were low (Table). Cataracts did not progress beyond

Progression of Cataract in the Diabetic Rat

Average Time in Weeks Required to Develop 1+ and 4+ Cataracts

Blood Sugar, Mg./100 Ml.	Weeks Required for 1+ Cataract	Weeks Required for 4+ Cataract	Interval Between Development of 1+ and 4+ Cataract
100-200	20 (1)*	.....	...†
201-300	17 (2)	.....	...†
301-400	8.8 (16)	14 (3)	5.2
401-500	5.1 (20)	13 (9)	7.9
501-600	5.1 (22)	15 (7)	8.9
601-700	4.9 (18)	12 (3)	7.1

* The numeral in parentheses is the number of surviving animals. The progression of each group from 1+ to 4+ varies, as some of the animals were either killed for histologic study or died.

† Five animals in each group went 20 weeks without any sign of cataract.

the 1+ stage in any animal with blood sugar levels below 300 mg/100 ml. Furthermore, lens opacities did not appear in 10 animals that had blood sugar levels between 100 and 300 mg/100 ml. and were followed for 20 weeks.

There was a negligible difference in the rate of cataract formation in the animals fed a stock diet and those fed a stock diet to which was added 0.1% nicotinic acid (Fig. 1*A*). Both groups had developed a 1+ cataract by the eighth week. This was a longer period of time than was required in the average diabetic rat, but was attributed to their lower food intake. The control rats did show some delay in the progression of cataract, but, as seen in the graph, the blood sugar levels of these animals were somewhat lower.

Cysteine apparently did not offer any protection against the development of diabetic cataract. As shown in Figure 1*B*, the rate of cataract formation in the cysteine-treated group of animals was essentially the same as in the untreated group. The cysteine group had developed a 1+ opacity by the seventh week and the untreated animals by the eighth week. In spite of the lower blood sugar levels of the cysteine group, the progression of cataract formation was very nearly the same.

Furthermore, no significant difference was found in the cataract progression of 12 diabetic rats maintained in the chamber with

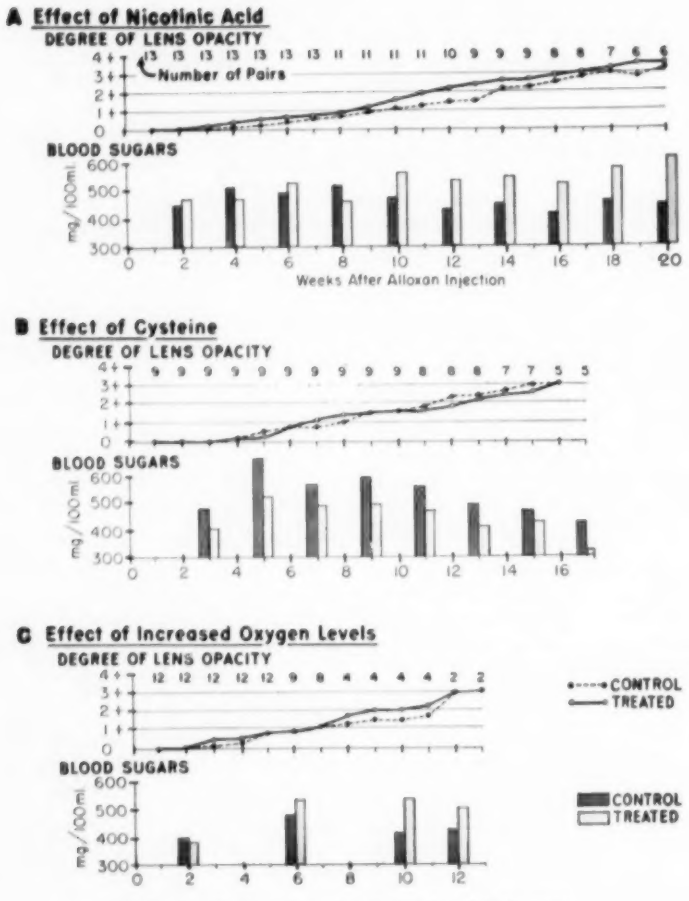

Fig. 1.—Progression of cataract in diabetic rats.

A, effect of nicotinic acid. In spite of the slightly higher blood sugar levels of the nicotinic acid group, the rate of cataract formation was very nearly the same as that found in the diabetic controls. *B*, effect of cysteine. The control group had the higher blood sugar levels. Again, the rate of cataract formation was very similar in the two groups. *C*, effect of increased oxygen concentration. The animals in the oxygen chamber had consistently higher blood sugar levels, with a somewhat faster rate of cataract formation. However, after the seventh week only four pairs of animals remained, not enough for a statistical analysis.

increased oxygen levels, as compared with "control" diabetics (Fig. 1*C*). Both groups had developed a 1+ opacity by the seventh week. The cataracts in the animals in the oxygen chamber seemed for a time to progress more rapidly than the controls, but by the 12th week the average cataract formation was similar in the two groups. The variation in these two groups after the seventh week is not significant, since only four pairs of animals were surviving at this time.

B. Rabbits.—In rabbits, the first lens change was seen in the equatorial region as either fine, radiating, hair-like opacities or vacuoles. Of 25 diabetic rabbits, all but 7 developed these opacities within the first week after alloxan injection. In two animals the opacities did not develop until the second week after injection; in three animals three

weeks was required, and in two animals lens opacities did not appear until the fourth week following injection.

After the equatorial region of the lens showed vacuoles, the opacities spread toward the central part of the lens. These new peripheral opacities consisted of vacuoles and spokes. Usually they became continuous, but occasionally two distinct zones of opacities were noted. This condition appeared in the more severely diabetic animals as early as the third week following injection of alloxan. Later, about the seventh week, changes were seen in the posterior subcapsular region, usually in the form of a plaque-like opacity. In three animals with blood sugars varying between 188 and 291 mg/100 ml. the cataract did not progress beyond this stage. In only two animals were distinct anterior subcapsular changes seen. Following the central anterior or posterior subcapsular changes, the opacities spread into the anterior and posterior cortex, causing the lens to become opaque. This occurred as early as 12 weeks in the more severely diabetic animals.

In five additional diabetic animals initial equatorial opacities were noted in the lens, but as the animals became less diabetic these opacities cleared. However, once a posterior subcapsular opacity had developed, even though the animals became less diabetic, there appeared to be no reversal of the opacity.

II. HISTOLOGIC OBSERVATIONS

A. Rats.—Seven lenses were studied which did not have ophthalmoscopic signs of cataract. However, when they were examined histologically, four showed evidence of cataract. In this group of animals the duration of the diabetes varied from 6 weeks (blood sugar average, 498 mg/100 ml.) to 15 weeks (blood sugar average, 198 mg/100 ml.).

Twenty-seven rats with a 1+ cataract that had been present from 4 days to 12 weeks had a variety of histologic changes which were confined to the more superficial portion of the lens. In these animals the duration of the diabetes varied from 6 to 22 weeks; the individual average blood sugar levels, from 175 to 769 mg/100 ml.

The lenses of 10 rats that had been diabetic from 8 to 20 weeks and had a 2+ cataract were examined histologically. The individual average blood sugar levels varied from 348 to 663 mg/100 ml. These cataractous changes had been observed to be present from 3 to 14 weeks.

Five rats that had been diabetic from 10 to 34 weeks and had a 3+ cataract were studied. The individual average blood sugar levels varied from 322 to 509 mg/100 ml. Cataractous changes had been observed from 6 to 28 weeks. In general, this group of lenses showed more involvement of the deeper fibers than the 1+ and 2+ groups.

The lenses of 13 rats with a 4+ cataract were examined histologically. The individual average blood sugar levels varied from 371 to 504 mg/100 ml.; the duration of the diabetes, from 13 to 23 weeks. Cataractous changes had been present from 7 to 26 weeks. These lenses, of course, showed the most extensive changes of all lenses studied.

In the lenses of alloxan diabetic rats it was observed that equatorial changes were more pronounced in the group with a 1+ cataract and decreased in extent as the cataract became more marked. These equatorial changes consisted of disarrangement and degeneration of the nuclei of the lens fibers. Some fibers had vacuoles and were swollen (Fig. 2*B*). Also, degeneration of the nuclei in the cells of the lens epithelium was more pronounced in the 1+ group (Fig. 2*A*). In some instances the layer of epithelium was replaced by fluid (Fig. 2*C*).

With the exception of epithelial hyperplasia (Figs. 3*D*, *E*), which was almost the same in all groups, the remaining histologic changes became more pronounced as the ophthalmoscopic signs increased. The incidence of vacuoles and swollen fibers under the epithelium (Figs. 2*A*, 3*A*) or between the epithelium and capsule (Fig. 2*D*) increased from 51%, in the 1+ cataract group, to 77%, in the 4+ cataract group. The incidence of vacuoles and swollen fibers under the posterior capsule (Fig. 3*B*, *C*) increased from 53% to 100% in the 4+ group. Fragmented nuclei in the posterior region (Fig.

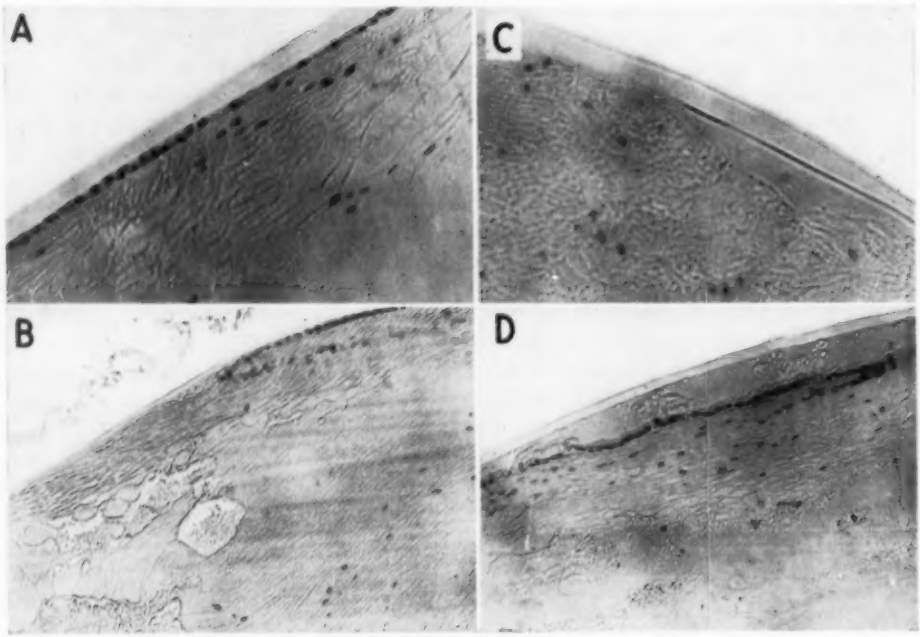

Fig. 2.—*A* (Rat 51, O.S.), no evidence of cataract. Blood sugar 235 mg/100 ml. Length of diabetes seven weeks. Section shows early subcapsular degeneration of the lens fibers, as evidenced by vacuoles and swollen fibers. Reduced 10% from mag. × 300.

B (Rat 7, O.D.), 4+ cataract. Blood sugar average 412 mg/100 ml. Length of diabetes 14 weeks. Section shows degeneration of the fibers in the deeper equatorial region, with large vacuoles with granular fluid and swollen fibers. The fibers nearest the capsule appear normal. Reduced 10% from mag. × 170.

C (Rat 30, O.S.), 4+ cataract. Blood sugar average 414 mg/100 ml. Length of diabetes 32 weeks.-Section shows degeneration of the epithelial cells with replacement by fluid. Below this is seen degeneration of the cortex with large, irregular fibers and degenerating nuclei. Reduced 10% from mag. × 300.

D (Rat 19, O.S.), 2+ cataract. Blood sugar average 488 mg/100 ml. Length of diabetes 18 weeks. Section shows large areas of fluid between the epithelial cells and the capsule. Immediately under the epithelium the cells are essentially normal. In the deeper cortex there is a marked disarrangement of fibers with large fluid areas, vacuoles, and fragmented nuclei. Reduced 10% from mag. × 170.

3C) were present in 100% of the 4+ cataract group, whereas they were present in only 19% of the 1+ group. Fragmented nuclei in the anterior region (Figs. *2C*, *3A*) and involvement of the deeper cortex, with the appearance of vacuoles, granular precipitation, swollen fibers, and fragmented nuclei, were present only in the 3+ and 4+ cataract groups. An almost complete involvement of the deeper cortex, with loss of homogeneity and disorganization, was present only in the 4+ cataract group.

In each group of animals having 1+ to 4+ cataracts, the blood sugar average, duration of diabetes, in weeks, duration of 1+ cataract, and the specific histologic findings were tabulated. The histologic changes did not correlate with any of the other three factors; that is, essentially the same histologic findings were present in a 1+ cataract whether it was an animal with a blood sugar of 180 mg/100 ml., in which a cataract had been present for 12 weeks, or in an animal with a blood sugar of 769 mg/100 ml., with a cataract of 2 weeks' duration.

B. Rabbits.—The lenses from 18 rabbits were studied histologically. In two of these animals there was no ophthalmoscopic evi-

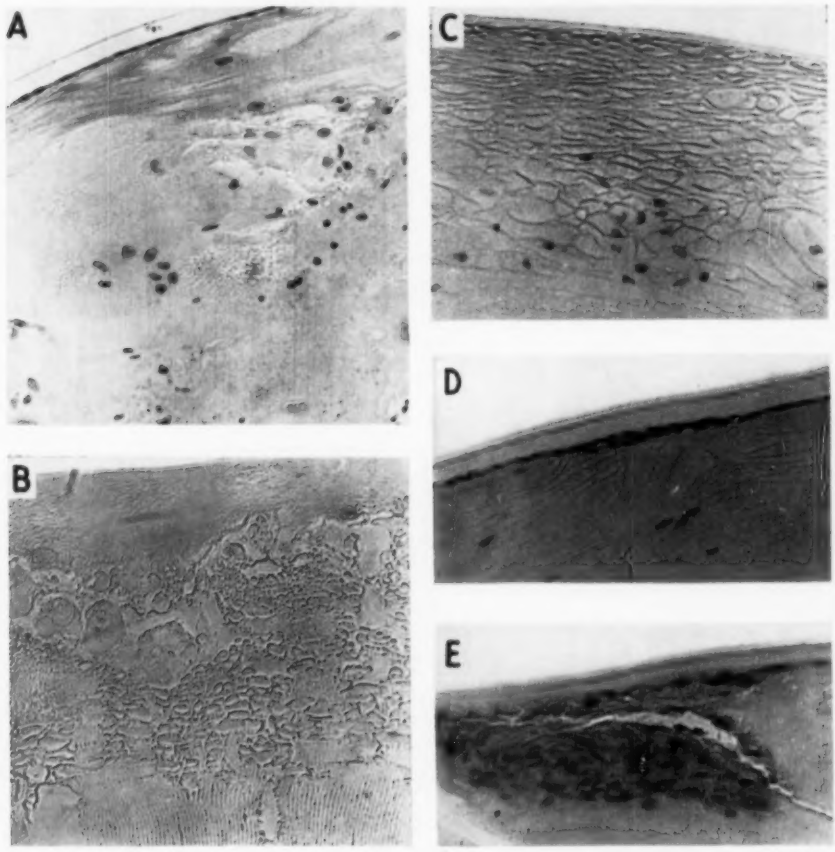

Fig. 3.—*A* (Rat 48, O.S.), 2+ cataract. Blood sugar average 406 mg/100 ml. Length of diabetes 15 weeks. Section shows large swollen fibers, deposition of granular fluid, and both large and small fragmented nuclei. Reduced 16% from mag. × 285.

B (Rat 28, O.S.), 4+ cataract. Blood sugar average 371 mg/100 ml. Length of diabetes 13 weeks. Section through posterior region, showing large swollen fibers and large vacuolated areas. Reduced 16% from mag. × 150.

C (Rat 62, O.S.), 1+ cataract. Blood sugar average 517 mg/100 ml. Length of diabetes nine weeks. Posterior region, showing large swollen fibers and large and small fragmented nuclei. Reduced 16% from mag. × 330.

D (Rat 55, O.D.), 1+ cataract. Blood sugar average 324 mg/100 ml. Length of diabetes 15 weeks. Section through anterior region, showing initial hyperplasia of the epithelium and swollen fibers beneath the epithelium. Reduced 16% from mag. × 330.

E (Rat 129, O.D.), 2+ cataract. Blood sugar average 478 mg/100 ml. Length of diabetes eight weeks. Section through anterior region, showing marked hyperplasia of the epithelium with formation of plaque. Cortical fibers have been replaced by fluid. Reduced 16% from mag. × 330.

dence of cataract, and in one the lens was histologically clear.

In all rabbit lenses studied the most consistent changes were equatorial vacuoles and swollen fibers (Fig. 4*A*, *B*), which were found in 34 of the lenses. The next most consistent finding was disarrangement of the nuclei in the equatorial region (Fig. 4*B*). This condition was present in 30 lenses. Vacuoles and swollen fibers were found in 26 lenses, and fragmented nuclei were found under the posterior capsule in 20 lenses. Eighteen lenses showed vacuoles and swollen fibers under the epithelium (Fig. 4*C*, *E*).

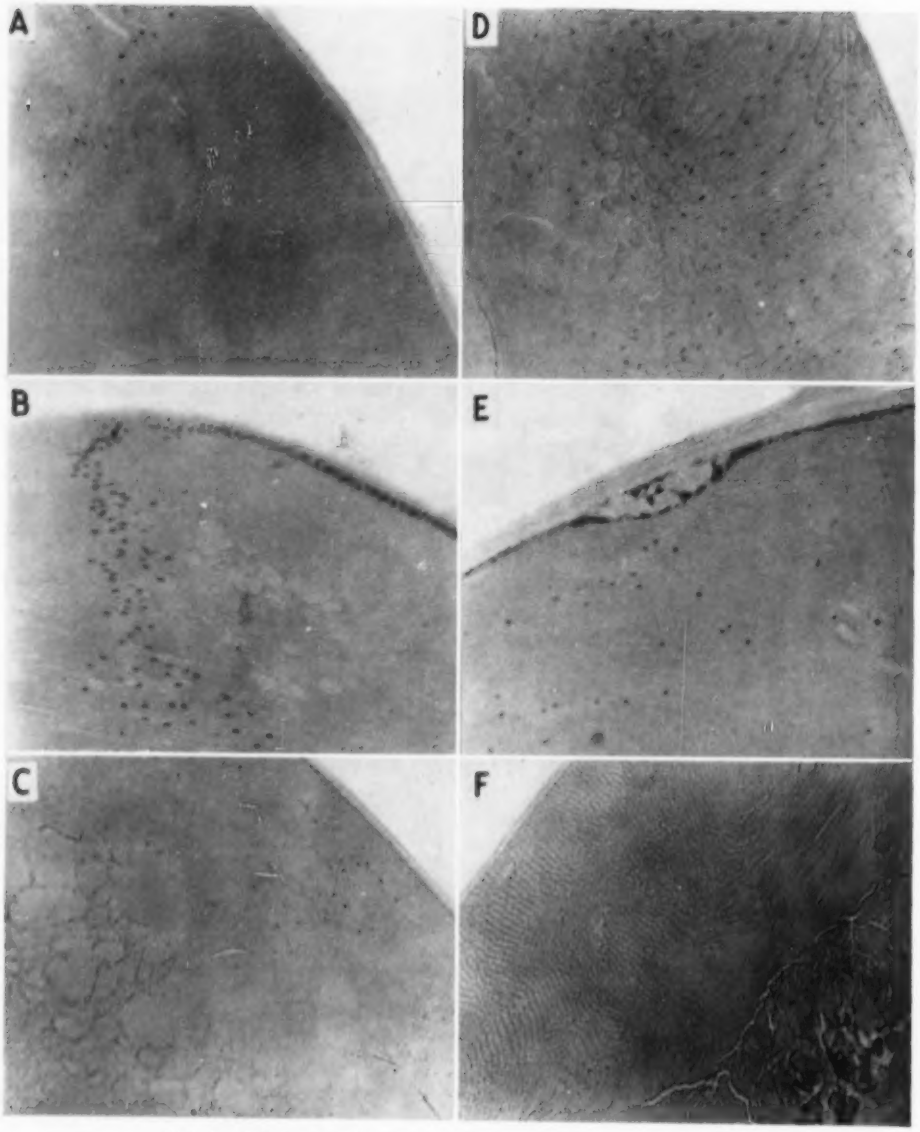

Fig. 4.—All photographs have been reduced 11% from mag. × 150.

A (Rabbit 19, O.S.), blood sugar average 327 mg/100 ml. Section just posterior to the equator, showing disarrangement of nuclei in the equatorial region with some degeneration of nuclei, swollen fibers, and vacuoles.

B. (Rabbit 4, O.D.), blood sugar average 265 mg/100 ml. Section at equatorial region which shows disarrangement of nuclei with swollen fibers and vacuoles.

C (Rabbit 18, O.D.), blood sugar average 494 mg/100 ml. Anterior section showing extensive, deep changes. Epithelium and fibers immediately under the epithelium appear normal. Deeper cortex shows fragmented nuclei. Still deeper are seen large areas containing fluid.

D (Rabbit 16, O.S.), blood sugar average 488 mg/100 ml. Tangential section at equator, which shows swollen fibers and deposition of fluid.

E (Rabbit 28, O.D.), blood sugar average 486 mg/100 ml. Anterior section showing hyperplasia of the epithelium, and the epithelium is displaced by fluid. In deeper areas vacuoles are seen.

F (Rabbit 28, O.S.), blood sugar average 486 mg/100 ml. Posterior region shows a few vacuoles superficially. In deeper areas there is a marked disturbance of the cortex and nucleus.

Pyknosis or fragmentation of the nuclei was found in the equatorial zone or in the epithelial cells in 12 lenses (Fig. 4*B*, *D*). In six lenses there was hyperplasia of the epithelium (Fig. 4*E*). Four lenses showed fragmented nuclei in the anterior subcapsular region (Fig. 4*C*, *E*). In two lenses there was a disruption and loss of homogeneity in the deeper cortex and nucleus, as shown in Figure 4*F*. In the animals with decreasing blood sugar levels the fibers in the superficial regions appeared to be assuming a normal structure.

COMMENT

From the present study it appears that there is direct relationship between the blood sugar levels and the development of a diabetic cataract. However, the present results do not indicate a definite arithmetic progression, as shown by Patterson.[14]

Contrary to a previous publication,[1] the addition of nicotinic acid to a stock diet did not appear to alter the incidence or progression of diabetic cataract in rats in a well-controlled experiment. If such animals are not pair-fed, even though they have similar blood sugar levels initially, the progression of cataract is decreased in the nicotinic-acid group, owing to their lower food intake and accompanying lower blood sugar levels.

Furthermore, once rats were made diabetic with alloxan and studied under pair-fed conditions, the injection of cysteine did not offer any protection against the development or progression of cataract. Moreover, the continuous exposure of rats to 38%-40% atmospheric oxygen did not materially alter the development of an experimental diabetic cataract.

In this study of cataract in the alloxan diabetic rat, the first opacity usually observed ophthalmoscopically was either in the anterior or in the posterior subcapsular region. Similar observations were made by Christenberry and Furth[7] in neutron cataracts in mice. Cogan, Goff, and Graves[8] found that in cataracts induced by radiation with fast neutrons in rabbits, vacuoles first developed at either end of the posterior suture and then extended toward and across the midline to form a rope of vacuoles just anterior to the posterior capsule. The first lens change observed ophthalmoscopically in the alloxan-diabetic rabbit, however, in contrast to the finding in the rat, always appeared first at the equator. This change consisted of fine linear opacities or vacuoles. Similar changes have been reported in the lenses of alloxan-diabetic rabbits by Bellows and Shoch[9] and, more recently, by Naidoff, Pincus, Town, and Scott.[10] Equatorial changes were found to be the earliest change in radiation cataract in rabbits by Leinfelder and Kerr,[11] von Sallmann,[12] and Alter and Leinfelder.[13]

In both rats and rabbits made diabetic with alloxan, one might logically expect the first change to be in the equatorial region of the developing lens fibers, as this area should be the most sensitive to abnormal metabolism. Histologic examinations have revealed that this was the case in both rats and rabbits.

Histologic abnormalities in rats and rabbit lenses may be summarized as follows: Initially there is a change in the equatorial region consisting of swelling of the fibers and the separation of the fibers by small and large vacuoles. The nuclei of the cells of the growing fibers are disarranged, and some nuclei show evidence of degeneration. Some of the swollen fibers beneath the epithelium, and also beneath the posterior capsule, degenerate.

With more advanced injury, there is further degeneration of the fibers of the cortex, and fragmenting nuclei are found in the anterior or posterior subcapsular region.

In the more severely diabetic animals a granular-like precipitation is found either between the epithelium and the capsule, replacing the epithelial cells, or under the posterior capsule. With still more advanced injury the deeper cortical fibers become swollen and vacuolated and have pyknotic nuclei.

In about half the lenses studied, particularly in those having 1+ or 2+ cataracts, the epithelial cells did not appear damaged. The earliest change observed in the epithelium was alterations in the nuclei, some

of which were pyknotic and others large and pale-staining. Later there was a complete loss of some nuclei. In some lenses with still more advanced stages of cataract formation there was a hyperplasia of the epithelium.

Some improvement of cataractous changes was observed in certain diabetic animals, when there was a gradual lowering of the blood sugar levels. This improvement, which occurred first in the equatorial zone, was confined chiefly to the superficial fibers.

From this study, it appears that the histologic changes in the lenses are not always directly correlated with the blood sugar levels found in the diabetic animal. Furthermore, there is very little relationship between the length of diabetes, in weeks, or the duration of 1+ cataract as compared with the histologic changes. This would suggest that a factor other than the high blood sugar per se, probably the defective utilization of glucose, is responsible for the injury to the lens.

SUMMARY

An ophthalmoscopic and histologic study of cataracts caused by alloxan diabetes in the rat and rabbits is presented. The formation and progression of diabetic cataract are observed in relation to the severity of the diabetes. The addition of nicotinic acid, the injection of cysteine, and the maintenance of rats in an atmosphere of 38%-40% oxygen were not found to alter the progression of a cataract in alloxan-diabetic rats.

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Control of Experimental Herpes Simplex Virus Keratitis

Hormonal, Vaccine, and Antibiotic Therapy

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With the Technical Assistance of S. Inwald and M. McCleary

The treatment of experimental herpes simplex virus keratitis with cortisone or corticotropin locally or systemically has been uniformly unfavorable or ineffective in the experience of several groups of investigators.* This is not peculiar to the herpes simplex virus, because the same undesirable results have occurred in the treatment of experimental poliomyelitis [5] and in Coxsackie,[6] influenza,† and mumps [8] virus infections. Presumably, these infections have a minimal hypersensitivity factor, since it is in the control of the latter that cortisone excels. Several other effects, however, on tissue resistance and immunity have been ascribed to cortisone. As Leopold [9] has pointed out, these actions are generally inhibitory in that there is a decrease not only in allergic responses but also in inflammatory responses to topical injury, in granuloma formation, and in resistance to certain infections.

According to Selye,[10] in distinct contrast to cortisone, the somatotropic hormone (STH), the so-called growth hormone, which is liberated by the anterior lobe of the pituitary as the result of tissue damage, stimulates allergic and inflammatory responses, granulation tissue formation, and resistance to infection. STH stimulates the adrenal cortex to produce mineralocorticoids, such as desoxycorticosterone, and also directly sensitizes connective tissue elements throughout the body to the actions of mineralocorticoids. By virtue of the latter quality, it should be reactive upon local or topical application to tissues.

Armed with this sufficiently attractive theoretical basis for the use of STH in the treatment of viral and, perhaps, other infectious diseases, and supplied with a small quantity of purified STH powder, through the courtesy of Merck & Co., Inc., we initially undertook this study to determine the effect of that hormone in experimental herpes simplex virus keratitis. The study was then broadened to include desoxycorticosterone and two of the other principal physiologically active steroids of the adrenal cortex, namely, androgenic and estrogenic substances. Also, in view of Griswold and Bowen's[11] successful clinical experiences in controlling herpes simplex infections with influenza vaccine, the effect of the latter upon the experimental corneal disease was studied. Finally, a new antibiotic (O. C. 1315-262, Sharp & Dohme), claimed to be of value in certain viral diseases, was also investigated.

METHODS

Corneal scrapings from a patient presenting a typical herpes simplex dendritic ulcer were directly deposited upon both corneas of a rabbit. Three superficial linear scratch incisions were made in the corneas immediately prior to inoculation. Four days later actively flourishing dendritic ulcers were present on the rabbit's corneas. Scrapings from

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From the Research Department, Wills Eye Hospital.

* References 1 to 4.

† References 7 and 8.

these lesions were suspended in a small quantity of gelatin-saline solution and transferred to the cornea and nictitating membrane of certain rabbits designated as reservoir animals to assure an adequate and ready supply of cornea-adapted virus.

In addition, the virus was made egg-adapted by propagation on the chorioallantoic membrane of the developing chick embryo. It was used in a dilution of 10^{-3} in the rest of the experiments for infection of the test and control rabbits' corneas. The choice of cornea-adapted or egg-adapted virus was purely one of convenience, for either source was found to produce the same response upon rabbit corneal inoculation. Care was taken, however, in any one series of experiments to use the same source of virus for the test and the control rabbits.

Both the test and the control corneas were observed with slit-lamp microscopy at fairly regular intervals. The lesions were stained with 1% methylene blue solution and recorded as to grade of severity, between 0 and 4, as previously described.[3] Except for the STH experiments, where insufficient hormone was at hand to treat a great enough number of infected rabbits, the grade of severity was averaged for each day of observation. The graphs based on these data readily indicate the results obtained in each instance.

SOMATOTROPIC (STH) HORMONE

Subconjunctival Injections.—One adult rabbit, after inoculation of both scarified corneas with cornea-adapted virus, received an injection of 0.5 cc. of saline containing 10 mg. of STH into the right superior bulbar conjunctival area. The left eye, as a control, received a similar subconjunctival injection of 0.5 cc. of saline alone. No further treatment was administered to either eye. Two days later both corneas were similarly involved, with a few scattered superficial dendritic ulcers. By the fifth day, the treated eye looked definitely improved; the control was worse. At the end of one week, however, both corneas presented similar involvement, with large, lake-like ulcers. From this point on the course of the treated eye was downhill, while the control slowly improved. At the end of two weeks the treated cornea was covered with large, lake-like ulcers; the control had only one small, subsiding similar ulcer. By the end of the fourth week the treated cornea presented a large vascularized opacity above and a still active confluent, lake-like ulcer below. The control at that time was practically fully recovered.

Apparently, a single large early subconjunctival injection of STH may have a transient inhibitory effect about the fifth day, but a prolonged and deleterious stimulating effect on the infection after the first week.

The corneas of four other rabbits were similarly prepared and inoculated with cornea-adapted virus. Two days later all corneas were approximately equally involved with scattered small dendritic ulcers. One eye of each animal then received 0.5 cc. of saline containing 5 mg. of STH subconjunctivally daily for four successive days. The opposite eye of each rabbit, as a control, received a similar subconjunctival injection of 0.5 cc. of saline alone. Two days later one of the treated eyes was much better than its control, and on the following day was completely clear of keratitis; the control continued to be quite severely involved. The three other treated eyes on the second day of treatment presented the same degree of involvement as the controls. On the third day the treated corneas were all worse than their controls, and this disparity in favor of the controls continued apace until the end of the first week, when observations were terminated.

The experiments so far indicate that the local injection of STH to prevent or treat established herpes simplex keratitis is of no value, and may even heighten the severity of the infection.

Drops.—The corneas of three rabbits were inoculated with cornea-adapted virus and were given two days for the development of approximately equal involvement, with scattered small dendritic ulcers. Treatment of each left eye was then instituted by instilling every hour during the day 1 drop of a saline suspension containing 1 mg. of STH in each cubic centimeter. The control (right) eyes received a drop of saline on the same schedule. This was continued for four successive days.

From the second day of treatment one treated eye was better than its control and was practically healed at the end of one week, while its control remained severely involved for two weeks. The second treated eye did

not differ from its control for one week; it then started to improve and was healed by the third week; the control was definitely worse during the second week but showed much improvement in the third week. The third treated eye was the same as its control for one week, and then the animal died, of unknown causes.

A group of six adult rabbits had inoculation of each cornea with egg-adapted virus. The following day treatment was instituted by the instillation of 1 drop of STH, 25 mg. per cubic centimeter of 1:5000 aqueous benzalkonium (Zephiran) chloride solution, in the right eye of each animal. The left eyes, as controls, were treated with 1 drop of 1:5000 aqueous benzalkonium chloride solution. Treatment was carried out every two hours during the day for three successive days.

From the second day on, definite, and in most instances marked, takes were observed in all eyes. Each daily record revealed a uniformly consistent result; i. e., the control eye in each animal appeared much less severely involved than the treated eye. Observations were terminated at the end of a week because of conclusive continued advantage in favor of the control eyes.

The treatment with drops at a low-dose level were rather inconclusive. Certainly, no marked early improvement resulted from the treatment. The impression was gained, however, that these eyes in general fared better than the injection-treated eyes. Obviously, treatment with the higher concentration of drops, as chosen, was ineffective.

Subcutaneous Injections.—Both corneas of six rabbits were inoculated with egg-adapted virus. Three of these animals received no treatment and served as controls. The other three rabbits were given a daily subcutaneous injection of STH for five successive days, starting on the day of corneal inoculation. One of these animals received a daily dose of 8 mg.; the second, a daily dose of 4 mg., and the last, a daily dose of 2 mg.

On the second day after inoculation the 8 and 4 mg. rabbits were definitely less severely infected than the controls, but the 2 mg. rabbit was worse than the controls. On the third day the 4 mg. rabbit still maintained its relative advantage, whereas all the other animals were equally and severely infected. By the fifth day all animals were equally and severely infected, and this picture remained unchanged at the end of the observation period, on the seventh day. It is apparent that systemic STH has little effect upon the development of the experimental corneal lesions other than perhaps a transient retardation about the second or third day.

Summary of STH Therapy.—Subconjunctival injection, prophylactically or after the lesions have been established 48 hours, failed to influence favorably the course of infection.

Drop treatment in low concentration after the lesions had been established 48 hours was slightly effective.

Drop treatment in higher concentration after the lesions had been established 24 hours was ineffective.

Prophylactic and concurrent subcutaneous injections have possibly a transient retarding effect up to the second or third day.

DESOXYCORTICOSTERONE (DOCA)

The preparation of hormone used was desoxycorticosterone acetate in propylene glycol and 20% alcohol, 10 mg. per cubic centimeter, prepared by the Schering Corporation under the name of Cortate. The herpes simplex virus used was the egg-adapted strain.

Intramuscular Injections.—The corneas of 12 rabbits were inoculated with the virus. Immediately afterward three animals were given an intramuscular injection of 1 mg. of DOCA; three other animals, 0.5 mg. of DOCA, and the remaining six animals were untreated, for controls. The intramuscular injections were repeated once daily for four more days in succession. The results are plotted in Figure 1, which shows remarkable similarity between the treated and the control animals.

Drops.—The corneas of 12 rabbits were inoculated with the virus. Immediate local treatment was instituted in the right eye of each animal. Six rabbits received a concen-

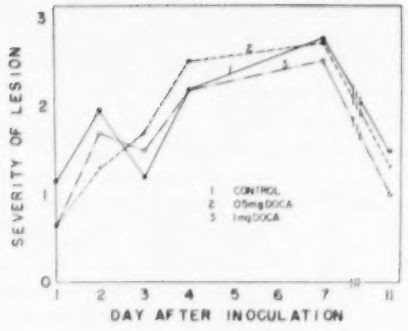

Fig. 1.—Influence of systemic DOCA on experimental herpes simplex keratitis in the rabbit.

2 mg. per cubic centimeter, and the other 6 animals a concentration of 10 mg. per cubic centimeter. Figure 3 shows that the results are no different from those obtained in the previous group.

Summary of DOCA Therapy.—Prophylactic and concurrent intramuscular injection or local drop therapy with desoxycorticosterone (DOCA) is ineffective in the control of experimental herpes simplex keratitis.

ESTROGENIC HORMONE

Fourteen adult rabbits, equally divided as to sex, were each given an intramuscular in-

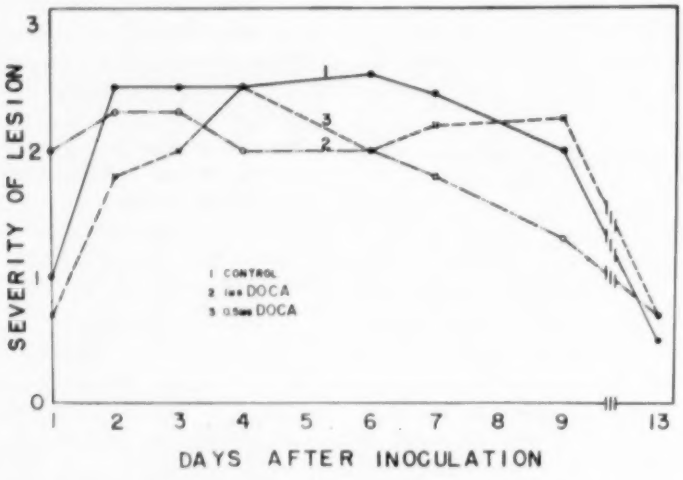

Fig. 2.—Influence of DOCA drops on experimental herpes simplex keratitis in the rabbit.

tration of 0.5 mg. of DOCA per cubic centimeter, and 6 received a concentration of 1 mg. of DOCA per cubic centimeter. Two drops were instilled every three hours for four applications during the day. Treatment was maintained for five successive days. The left eyes, receiving no treatment, constituted the controls. The results as plotted in Figure 2, again, show no significant difference between the treated and the control eyes.

Another group of 12 rabbits was similarly inoculated and treated, except that 6 animals received DOCA drops in a concentration of

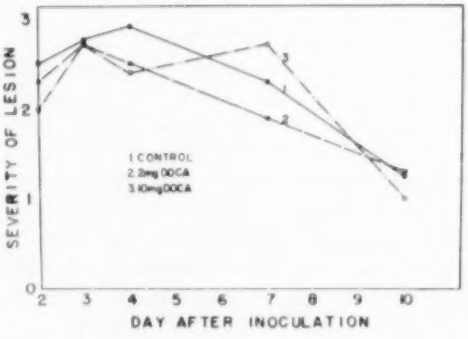

Fig. 3.—Influence of DOCA drops on experimental herpes simplex keratitis in the rabbit.

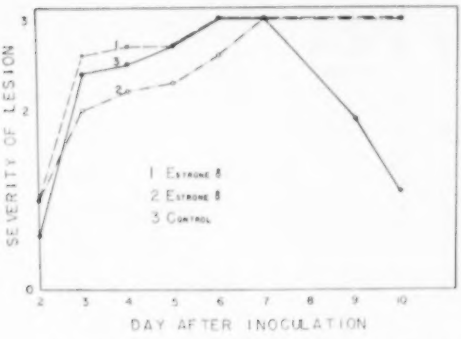

Fig. 4.—Influence of systemic estrone on experimental herpes simplex keratitis in the rabbit.

jection of 0.5 mg. of crystalline estrone (Lilly) suspended in isotonic saline solution. The injections were given once daily on the fifth and the third day prior to and the second day after inoculation of their scarified corneas with cornea-adapted herpes simplex virus. As controls, 11 other adult rabbits had similar inoculation of their scarified corneas.

The results, as plotted in Figure 4, show no significant difference between the control and the treated eyes for the first week. After that the controls started to improve, but the treated eyes remained severely infected.

Obviously, prophylactic and concurrent estrogenic hormone therapy has no beneficial effect on experimental herpes simplex virus keratitis.

ANDROGENIC HORMONE

Fourteen adult rabbits, equally divided as to sex, were each given an intramuscular injection of 6.25 mg. of crystalline testosterone (Lilly) suspended in isotonic saline solution. The injections were given once daily on the fifth and third days prior to and the second day after inoculation of their scarified corneas with cornea-adapted herpes simplex virus. Since this experiment was done at the same time as that on the estrogenic hormone group, results for the same 11 control animals used in that group were considered applicable to this group.

The results, as shown in Figure 5, differ in no way from the results obtained with estrogenic hormone for the first week. After that the male rabbits remained severely infected, but the female rabbits kept pace with the thenceforth rapidly improving controls.

The conclusion can only be drawn that prophylactic and concurrent androgenic hormone therapy has no therapeutic value in experimental herpes simplex keratitis.

INFLUENZA VACCINE

Eighteen adult rabbits were divided into three equal groups. The first group of six

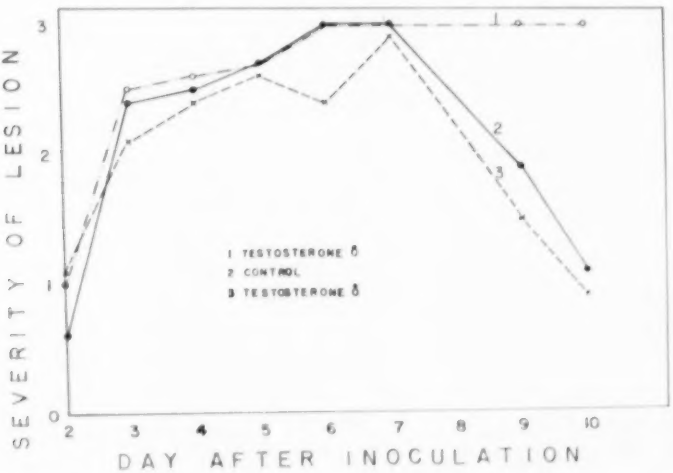

Fig. 5.—Influence of systemic testosterone on experimental herpes simplex keratitis in the rabbit.

animals, received 0.2 cc. of polyvalent influenza virus vaccine (Parke, Davis & Company) intramuscularly on the 14th and 13th days prior to corneal inoculation with egg-adapted herpes simplex virus. The second group, of six animals, received 0.1 cc. of influenza vaccine intramuscularly on the day of and the first and third days after inoculation of their corneas with the egg-adapted herpes simplex virus. The last group, of six animals, had their corneas similarly inoculated, received no treatment, and constituted the control group.

The results are plotted in Figure 6 and show that the first, or prophylactic, group, fared better than the second, or concurrent, group up to the third day but no better than the controls. After the third day there seemed to be little difference in any of the three groups.

The conclusion is apparent that prophylactic or concurrent influenza virus vaccine treatment is quite ineffective in experimental herpes simplex keratitis.

O. C. 1315-262

Ten normal adult rabbits were inoculated on both scarified corneas with cornea-adapted virus. The right eye of each animal was treated with 2 drops of O. C. 1315-262 solution at 15-minute intervals for four hours. The left eyes were kept as controls and received no treatment. Five of the right eyes received their first treatment immediately after inoculation, and the other five received their first treatment four hours after inoculation.

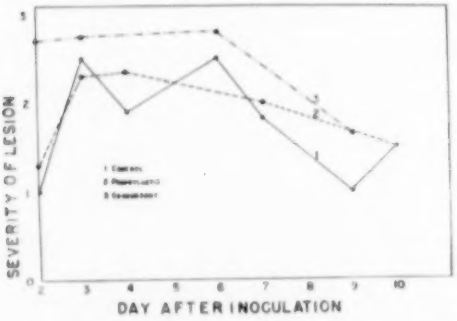

Fig. 6.—Influence of influenza vaccine on experimental herpes simplex keratitis in the rabbit.

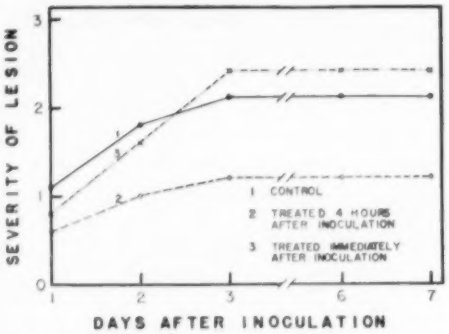

Fig. 7.—Influence of O. C. 1315-262 (Sharp & Dohme) on experimental herpes simplex keratitis. Inoculated corneas were treated at 15-minute intervals for 4 hours.

The results are plotted in Figure 7 and show no significant difference between the controls and the treated eyes.

The conclusion must be drawn that O. C. 1315-262 is of no value in experimental herpes simplex keratitis.

SUMMARY

In the treatment of experimental herpes simplex virus keratitis in the rabbit the following results were obtained:

1. Somatotropic hormone by subconjunctival injection and applied topically was ineffective, but prophylactic and concurrent subcutaneous injections had a possible transient retarding effect up to the third day of infection.

2. Desoxycorticosterone in prophylactic and concurrent intramuscular injections or in topical applications was ineffective.

3. Estrogenic or androgenic hormone injections given prophylactically or concurrently had no effect.

4. Influenza virus vaccine injections given prophylactically or concurrently had no effect.

5. O. C. 1315-262 solution (Sharp & Dohme) had no beneficial action.

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Variations in Morax-Axenfeld Diplobacillus

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In a recent communication on diplobacillary keratitis, we [1] commented upon the variable morphology of the Morax-Axenfeld Gram-negative diplobacillus (Moraxella lacunata). We have had an unique opportunity to demonstrate this remarkable, and hitherto unstressed, variability, and we hope that our findings will help to explain some of the discrepancies found in the literature. Through the courtesy of Dr. Y. Mitsui, of Kumamoto, and Dr. Seymour Halpert, of New York, we were able to obtain a culture of the Gram-positive micrococci described by Mitsui and his colleagues [2] as etiologic agents in angular conjunctivitis.

In our laboratory, we noticed accidentally that when a culture of the Morax-Axenfeld bacillus was left out for five days the microscopic appearance of the organisms changed. The bacteria became much shorter and tended to lose their Gram-negative staining characteristic. Moreover, they tended to retain these new characteristics on subsequent blood-agar transfers. An interesting example of this was seen in a case in which a young woman had suffered from unilateral conjunctivitis for years. Smears showed only a single Gram-negative diplobacillus, and on culture there was no growth on blood agar or Löffler's serum. Because of another project going on simultaneously, we happened to make egg inoculations of the conjunctival exudate. Organisms grew, and were seen to be Gram-negative bacilli, mostly in the typical diplobacillary form, but also in filamentary and club-shaped forms. A subculture on blood agar produced an abundant growth of the Morax-Axenfeld diplobacillus. Here, there were no filamentary or club-shaped forms. This culture was then left on a table for five days, and growth continued at room temperature. Smears made at that time showed a preponderance of cocci, mostly Gram-positive, although some were Gram-negative. The cocci were arranged like Staphylococcus clusters. These were again transferred to yolk sacs, whereupon the diplobacillary forms reappeared, accompanied by club-shaped and filamentary variants.

A great stimulus to our curiosity about this phenomenon was the frequent finding of the odd Gram-positive diplobacillus, sometimes in a V-form, found in some smears of Morax-Axenfeld cases, and the not infrequent presence of a Gram-negative bacillus in Corynebacterium xerose cases. However, it was the paper by Mitsui, Hinokuma, and Tanaka [2] that gave us the opportunity to investigate this apparent discrepancy. In their very interesting experiments, they inoculated the conjunctival and skin surfaces of the lids of human volunteers with pure cultures of the Morax-Axenfeld diplobacillus. Despite their precautions, they found in their "takes" an unidentified Gram-positive Micrococcus along with the diplobacillus. The thought occurred to us that this might be a variant form of the diplobacillus; accordingly, we requested a sample culture from the authors. This was obtained through the courtesy of Dr. Halpert, and we proceeded to do a series of transfers, as shown in the accompanying pictures. The media used were blood agar, plain agar, serum broth, Löffler's medium, ascitic serum, plain broth, and yolk sacs.

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From examination of the evolved specimens, it was apparent that continued culture on blood agar encouraged the development of the Gram-positive micrococcal form and continued culture on liquid media encouraged the development of the Gram-negative diplobacillary form. Continued manipulation could make the organism take on either appearance, as desired. Not only was there a marked variety in form, but the specimens ran the whole gamut of color, varying from an almost invisible pale pink to an intense dark blue. In some cultures capsules could be seen; in others, bloated forms; some liquid cultures produced long, coiled filaments, and in still others one could see cocci and bacilli in the same field, or Gram-positive and Gram-negative forms in the same field. To be certain that none of this was due to contamination or error, an added precaution was taken. On the appearance of

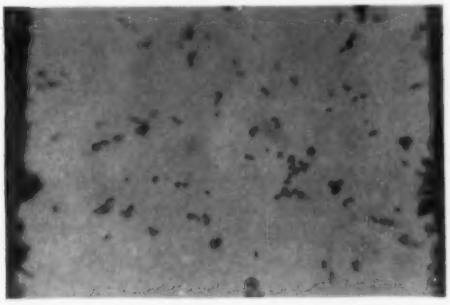
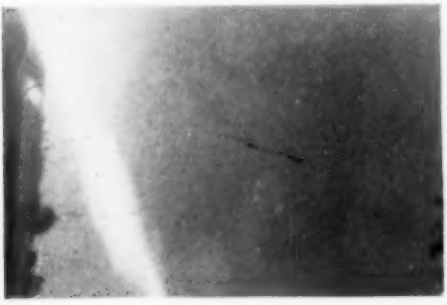


Fig. 1.—Gram-positive cocci.

Fig. 2.—Typical Gram-negative diplobacilli, direct descendants of the cocci in Figure 1.

Fig. 3.—Gram-negative rod chain, two members of which are typical, the remainder being bloated forms.



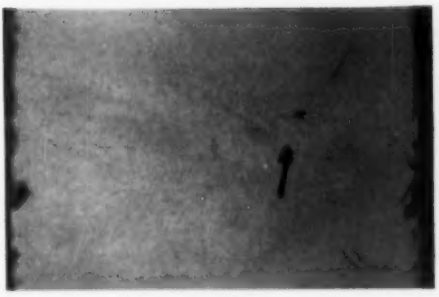


Fig. 4.—Gram-negative flask-shaped variant.

Fig. 5.—Gram-negative filamentary forms.

Fig. 6.—Gram-negative and Gram-positive diplobacilli in same chain, accompanied by other bizarre Gram-negative forms.

Fig. 7.—Gram-positive diplobacilli.

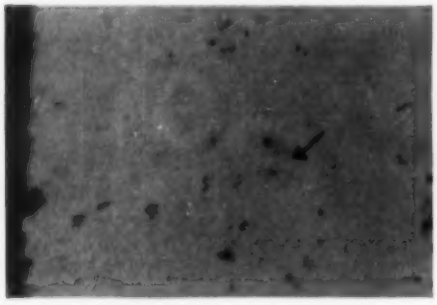
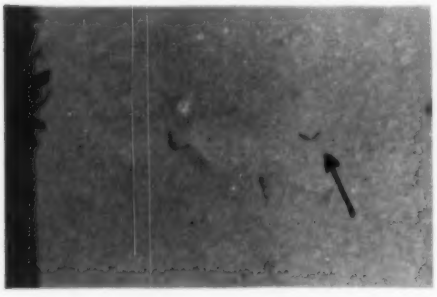


Fig. 8.—Metachromatic cocci, showing capsules.

Fig. 9.—Gram-positive diplobacilli, V-shaped like C. xerose, and other metachromatic forms.



a different form, either morphologically or in staining quality, the organism was cultured back to the preceding color and shape by the use of appropriate media. Typical examples of these many variations are illustrated in the accompanying photographs, taken from Kodachromes of the slides. The legends accompanying the figures are self-explanatory.

Axenfeld [3] noted that the power to liquefy gelatin and grow freely on agar, which was present at the outset in his two earliest examples of the Petit bacillus, was gradually lost in successive generations, so that the organism more nearly approached the Morax-Axenfeld type. McNab continued the research but found it impossible to develop in the Morax-Axenfeld bacillus the power of liquefying gelatin and of propagating it from bouillion to ordinary agar. This we have

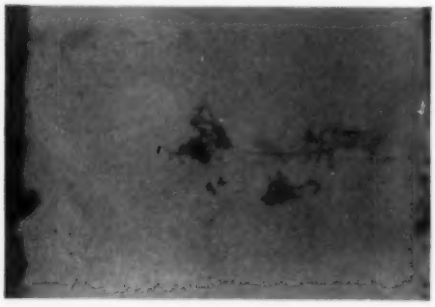
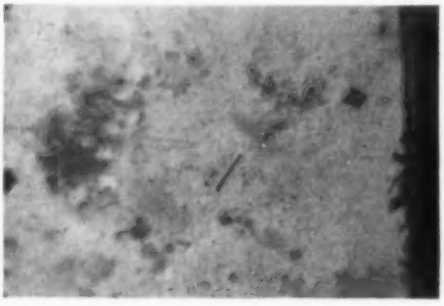


Fig. 10.—Gram-negative filamentary and coccal forms in same field.

Fig. 11.—Gram-negative granular bacilli, showing transition between cocci and bacilli.

Fig. 12.—Gram-positive, very large, rod-like anthrax bacilli.



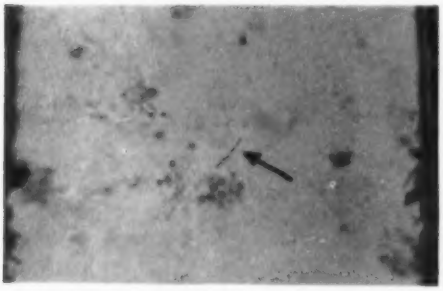


Fig. 13.—Gram-positive diplobacilli, very short.

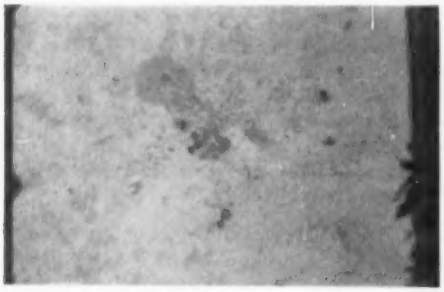


Fig. 14.—Gram-positive (metachromatic) diplobacillus, showing granules.

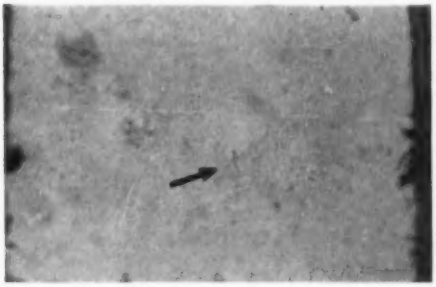


Fig. 15.—Gram-positive member and Gram-negative member on one diplobacillus.

Fig. 16.—Gram-positive short rods, encapsulated, on tissue culture.

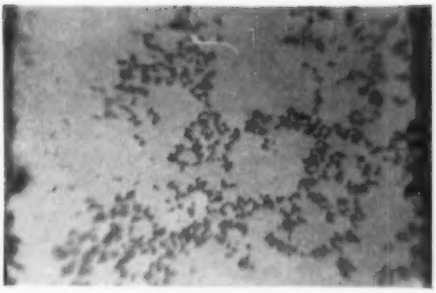
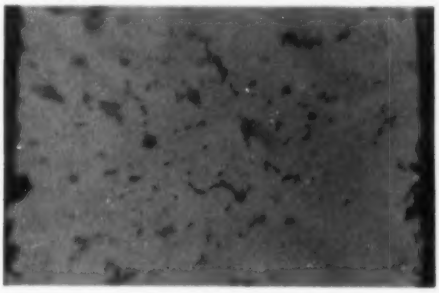


Fig. 17.—Metachromatic cocci.

Fig. 18.—Gram-negative bacilli, showing variations.

Fig. 19.—Metachromatic bacilli, showing variations.

Fig. 20.—Metachromatic coccobacillary forms.



been able to do in our laboratory, possibly because of hardier strains.

Collins and Mayou [4] noted that occasionally a vacant area could be found about the bacilli, suggesting the presence of a capsule. Such examples may be seen in our figures. They, too, noted that in serum broth involution forms could occur, but were not common. However, in blood serum long chains of organisms with some distorted bacilli appeared. That such variations can occur is a rather well-established fact in general bacteriology.

Dubos,[5] speaking of adaptation in bacteria stated:

> It is often possible to render a given bacterial culture more resistant to one or another substance, or to various inimical environmental factors such as high osmotic pressure, by transferring the culture into media containing progressively increasing concentrations of the toxic agent under consideration. Conversely, certain bacterial species which exhibit complex nutritional requirements when first isolated from pathologic material can be rendered less exacting by transferring them into media containing progressively lower concentrations, and finally none, of some growth factor which appeared essential at the beginning. . . . Bacteria can be modified not only with reference to their ability to grow in new media, but also in their behavior toward animal hosts . . . One can often control at will, by experimental procedures, the direction and extent of the adaptation, and for these reasons the phenomena are often described under the name of training.

He cites two theories to account for this: One is the quantitative alteration of the enzymatic constitution of the cell by the environment, and the other is the presence of a latent mutation quality in the organism which becomes manifest on environmental change. That these two theories are not entirely incompatible is apparent from the recent discovery of the importance of enzymes in the structure of genes.

Jordan and Burrows [6] stress the two main types of variation. One is the "fluctuating" type, which occurs in a certain percentage of reproductions, and may be described on a modal frequency curve. The other is the abrupt and well-marked "sport," or "mutation," type, in which there is a sudden change of characteristics, without gradual intervening forms. They note that large amounts of salt in the solution will produce bizarre forms; carbohydrate media may cause the production of capsules; temperature may determine the presence of flagellae, and spore formers may become asporogenous at some unsuitable temperatures or in the presence of dilute antiseptics. They also propose that toxins may be of great importance in these phenomena, including the toxins of aging and degeneration. Other known causes are x-rays, ultraviolet light, and certain drugs.

Zinsser [7] says:

> It has been suggested that the process of evolution in bacteria proceeded from autotrophic organisms, which must synthesize all their organic constituents including the enzyme systems from rock, water, and air, to the obligatory parasites, and finally to the viruses which may be dependent upon the enzyme systems of the host cells.

Experiments in transformed organisms show that the transforming agent perpetuates itself indefinitely in the transformed cell. "It remains to be seen whether transformation of this type can cross the boundary of species."

Topley and Wilson [8] noted the interesting fact that replacement of choline with ethanolamine will cause pneumococci to grow in characteristically long chains, and that in other cultures too little iron will give curved, elongated, atypical bacilli. They stress the importance of adaptive and constitutive enzymes, the former occurring when the substrate is present, and the latter whether it is present or not. They note that mutations are not equally rare in all bacterial strains, and that some produce variants so constantly and in such large numbers that they are often designated as "unstable" strains.

It is apparent, then, that remarkable variations in morphology, cultural characteristics, and biological behavior can occur in certain bacteria. Quite possibly it has been our failure to recognize these potentialities that has caused confusion in our clinical and bacteriologic approach. We must realize that the bacterium can masquerade under various guises, depending more or less upon the ma-

terials at hand, and can perform a versatile repertoire of noxious acts, depending more or less upon the opportunities at hand. If we are aware of this, we can reduce the the numbers of organisms in our classifications and can account for different clinical pictures caused by one organism. We now find it easier to believe that the same organism can cause both hypopyon keratitis and angular conjunctivitis, and that the classical differentiation into the Morax-Axenfeld bacillus and the diplobacillus of Petit is unnecessary. Also, in Mitsui's experiment we can now consider the possibility that micrococci did not develop spontaneously in the conjunctival sac, but that they developed from Gram-negative diplobacilli placed there.

In summary, our experiments indicate that the Gram-negative diplobacillus may be designated as unstable, since it can be made to assume a variety of forms, according to its environment. Variations of this organism are presented which are indistinguishable morphologically from the diplobacillus of Petit, the Micrococcus of Mitsui, C. xerose, the anthrax bacillus, Staphylococcus, and others. The speculations on the conjunctival flora which follow will be described in a later paper.

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Surgical Treatment of Congenital Palpebral Phimosis

The Y-V Operation

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A number of variations of congenital deformities are included under the heading of congenital palpebral phimosis. In general, the main problem is the narrow dimensions of the interpalpebral fissure, both horizontal and vertical. One of the main features is the wide intercanthal distance with various types of epicanthal skin folds at the medial canthus.

The child with this condition usually has a rather flat face, with very little of the normal depression at the medial canthus, and consequently little or no nasal bridge. In addition, he holds his head far back to be able to see out of the narrow interpalpebral fissures, which are little more than slits in severe cases. The ptosis is aggravated when the interpalpebral fissure is also short temporally. In milder cases the condition of the lateral canthi may be quite normal. There is almost always a lack of the transverse fold in the upper lid and a flattening of the normal depression under the highly arched brows, characteristic of severe ptosis. There is frequently a rounded fold of skin hiding the medial canthus and, in the more marked cases, a true, or inverted, epicanthal fold. Also, there is a lack of the normal canthal depression medially. In addition, the medial canthi are displaced temporally, so that they encroach on the eyes and may hide the entire medial scleral area. The distance between the two canthi is, therefore, disproportionately greater than the interpupillary distance. The position of the lower lacrimal punctum is sometimes displaced so far temporally that it is in a vertical line with, or even lateral to, the middle of the cornea.

In extreme cases, in addition to the soft-tissue deformity, a bony deformity exists which is characterized by the wide distance between the medial orbital walls. When this is very marked, the improvement that can be accomplished surgically is limited by this bony abnormality.

The entire facial expression is frequently one of idiocy, which the child's mentality usually belies, for these children are often rated high in their classes at school. Occasionally there is some mental retardation, but this is not the rule. If the condition has remained untouched until adolescence (Fig. 1*A*, *B*, *C*,), these developing children are usually most appreciative of any help that can be obtained, and their outlook on life usually changes for the better.

The abnormality sometimes occurs in several siblings. I have seen one family in which 11 siblings were affected, and another in which the father and two daughters were affected, while others in the same family were unaffected. Some cases apparently occur as isolated examples.

The surgical treatment of this condition is never entirely satisfactory. However, considerable improvement in appearance and function may be obtained in most cases by (1) elongating the lateral canthus, when necessary; (2) shifting the medial canthus nasally, and if possible posteriorly, with removal of soft tissue to recreate a medial canthal depression; (3) relocating the lower lacrimal punctum to a more nasal position; (4) resecting the levator palpebrae superioris to widen the interpalpebral fissure vertically, and, at the same time, (5) creating the transverse fold of the upper lid.

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Fig. 1.—Moderate case of congenital palpebral phimosis in a 17-year-old girl, showing wide intercanthal distance and typical ptosis, with high-arched brows and lack of fold in upper lid. This represents the maximum possible elevation of the upper lids in this case. (*A*) both eyes; (*B*) left eye; (*C*) right eye.

Many surgical procedures have been suggested for the correction of this condition, all of which include a canthotomy temporally when necessary, but for the correction of the deformity at the medial canthus and the ptosis a great many procedures have been devised. The purpose of this paper is not to review these variations but simply to describe the technique that has seemed to me most reasonable, and one that has produced much improvement in appearance and function in these unfortunate cases. About 15 cases have been operated on, using the technique that is described below, in two stages.

THE OPERATION

Stage 1.—Elongation of the horizontal length of the interpalpebral fissure.

(*a*) A canthotomy is performed at the lateral canthus. The elongation of the lateral canthus, when necessary, is easily accomplished by making a horizontal cut with heavy scissors. The conjunctiva is then united to the skin with a few sutures to prevent the contraction of the canthus later.

(*b*) At the same time, the correction of the deformity of the medial canthus is carried out. I believe that in most instances a Y-V type of procedure is best.

An incision the shape of a Y turned horizontally (Fig. 2) is made, with the stem of the Y extending from about the midline on the bridge of the nose, on a level with the canthus, to 3 to 4 mm. from the canthus. Here the incision separates into two arms, one passing below the medial canthus into the lower lid for a distance of 1.5 to 2 cm., and the other passing above into the upper lid. There is usually considerable bleeding in this dissection, but the soft fascia and fatty tissue medial to and above and below the canthus need to be removed so that a depression in this area may be formed. While this dissection is being carried out, it is well

Fig. 2.—Plan of horizontal Y incision for moving the medial canthus toward the midline.

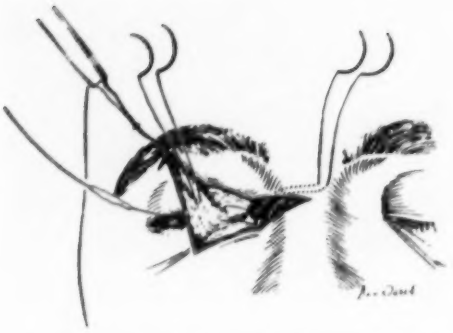

Fig. 3.—Plan of dissection and placement of two double-armed 4-0 chromic absorbable or nonabsorbable surgical sutures. Note lacrimal probe in lower canaliculus.

to identify the course of the lower lacrimal canaliculus by means of a probe passed into it, to avoid cutting it. The tissues around the medial canthus, down to and including the attachment of the medial canthal ligament, are dissected free, thus mobilizing this entire area. This dissection is carried down to the lower orbital margin, where the attachment of the tarso-orbital fascia is incised to free the lower lid. The incision must be carried far enough temporally to allow the canthus to be pulled nasally. For this purpose, the attachment of at least the medial part of the tarso-orbital fascia of the upper lid must also be incised along the upper orbital margin for a sufficient distance to allow the nasal portion of the upper lid to be stretched nasally. Unless this is done one will frequently find that the medial canthus cannot be moved very much. If the connection of the tarso-orbital fascia to the orbital margin above and below is palpated while the medial canthus is stretched nasally, one can readily tell whether it needs further separation to allow the canthus to be moved nasally.

A double-armed 4-0 chromic absorbable surgical (gut) suture with fairly heavy curved needles is then passed through the conjunctival surface of the medial canthus fairly near the skin and brought out medially near the canthal ligament (Fig. 3). Each needle then enters the periosteum on the medial wall of the orbit as far back as possible, being limited by the position of the lacrimal sac. These needles are then passed forward beneath the periosteum and emerge anteriorly through the skin near the midline above the level of the original incision.

Another, similar, double-armed suture is placed in the subcutaneous tissues about halfway along the arms of the Y and anchored similarly to the periosteum on the nasal wall of the orbit as far posteriorly as possible (Fig. 4). Both these sutures are tied over gauze or rubber pegs on the skin of the bridge of the nose. The triangle of skin and other tissue within the arms of the Y is thus advanced along the stem of the Y to form a V. This results in pulling the medial canthus nasally to lessen its encroachment on the medial aspect of the eye. The skin edges are united with fine, closely placed

Fig. 4.—Sutures passed as deep and posterior as possible, picking up the periosteum on the nasal wall of the orbit. Usually it is necessary to remove a triangle of skin (dotted area) at the base of each arm of the Y to allow for the migration of the flap nasally to form a horizontal V (after Burow's operation).

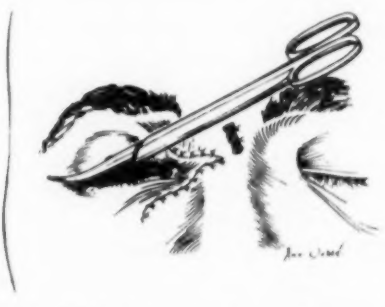

Fig. 5.—Final closure nasally—canthotomy temporally.

Fig. 6.—Same case as that shown in Figure 1 two months after Y-V operation on the right side and three weeks after operation on the left. Note the quite visible scars. Firm massage was started five weeks postoperatively.

6-0 nonabsorbable surgical (silk), or in children 6-0 chromic absorbable surgical, sutures (Fig. 5).

(*c*) When the location of the lacrimal punctum of the lower lid is displaced rather far temporally, it may need to be relocated. This can be done by passing a #3 or #4 probe along its course and dissecting out the canaliculus from the punctum for a suitable distance. A hole is then made on the inside of the medial canthus and the dissected canaliculus brought out through the conjunctival surface, in which a slit is made for a short distance at the proper site. The obliquely cut end of the canaliculus is then anchored to the margins of the conjunctival hole to preserve its opening. The protruding excess of the canaliculus is excised.

A pressure dressing is placed over the entire area, with a rather tightly wrapped gauze ball about the size of a marble (1 to 1.5 cm. in diameter) placed over the medial canthus to aid in the formation and retention of the depression in the region of the medial canthus. The dressing is changed at five- to seven-day intervals for a period of three weeks and the sutures removed at the second dressing.

There is some scarring along the line of the incision (Fig. 6), which flattens down to become barely visible in time (Figs. 7*A*, *B*, and *C* and 8). After four or five weeks it is well to start pressure massage over the scars, using a bland ointment, three times daily. In massaging these scars, the finger is placed on one part of the incision and the tissues are squeezed rather firmly between the ball of the

Fig. 7.—Same case as that in Figure 6, eyes open, one year later—five and three months after resection of levator on right and left upper lids, respectively. Note the more normal-appearing medial canthi, with the improved appearance of the upper lids, with palpebral folds. (*A*) both eyes; (*B*) right eye; (*C*) left eye.

finger and the underlying bone or other tissues, rubbing in a circular fashion. The purpose of this firm pressure is to break up the deep scar tissue along the lines of

Fig. 8.—Same case as that in Figure 7, with moderate closure of lids, as in sleep, showing a small chink, which is insufficient to produce any irritation from exposure. Note how the scars from the Y-V operations have practically disappeared.

the incisions in the deeper portions of the wounds. Surface stretching of tissues should be avoided to prevent stretching and widening of the skin scars.

Stage 2.—Operation for correction of the deformity of the upper lid.

(*a*) The best operation for the elevation of the lid is a resection of the levator. There is almost always sufficient power in the levator to produce good function in the lid. I prefer to do this through an external incision along the line of the future fold in the lid by the Eversbusch technique. The tendon of the levator is shortened and advanced, and some of the superficial tissue on the anterior surface of the tarsus is excised in order to keep the lid thin.

(*b*) The original incision is made along the site of the future fold, and, in closing, a continuous suture is used to close the skin and each bite picks up the deep tissue of the lid in order to anchor it down, thus producing the fold. It is maintained by a small special roll of gauze held in place by the pressure dressing.

Stage 1 on the two sides may be done at one sitting, although I believe it is better to do each stage of each side as a separate procedure, the first stage of each side being done first, and, subsequently, the ptosis on each side being cared for separately as well. The four procedures can be spaced at three-month intervals.

If a sufficient depression can be obtained at each medial canthus, nothing may need to be done for the bridge of the nose. Sometimes the bridge may have to be built up later if its development is seen to be grossly inadequate.

I do not want to leave the impression that I am fully satisfied with the results of this technique, but it has given greater improvement, and is less complicated, than most other techniques I have seen suggested.

SUMMARY

A technique for the lessening of the deformity met with in congenital palpebral phimosis is presented. The operation is designed to (1) elongate the fissure at the lateral canthus, (2) correct the fold of skin overhanging the medial canthus, (3) deepen or create a medial canthal depression, (4) move the medial canthus, and, if necessary, the lower lacrimal punctum, nasally, and at the same time create as much of a depression in this area as possible, and, at a second stage, (5) elevate and create a fold in the upper lid. In the bilateral correction of this condition, there are two stages at intervals of about three months for each eye.

The operation does leave scars, which, however, usually flatten out and leave very few residual marks.

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Iodopyracet (Diodrast) Injection for Orbital Tumors

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and
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Most roentgenologists will agree that iodopyracet (Diodrast) should be the safest radiopaque medium for retrobulbar injections.* It is commonly used in cardiography and pyelography. However, one should keep in mind the dangers of this compound, which contains 50% iodine.[1] Venospasm, sclerosing lipogranuloma, tissue slough, and occasional deaths have been reported with its use in various branches of medicine.†

In 1952 we performed experiments with orbital injection of iodopyracet in animals.[4] The dye produced excellent outlines of the muscle cone, Tenon's episcleral space, and the peripheral surgical space of rabbits. Neoprene latex tumors could be easily diagnosed and localized with these orbitograms. However, there resulted frequent injury to the eye and orbital tissues from such injections.

One cubic centimeter of 35% iodopyracet in the muscle cone of rabbits (roughly comparable to 5 cc. in man[5]) appeared to be the best amount for clear visualization of the normal structures. In four instances serial roentgenograms made at 30-minute intervals revealed only moderate absorption of the medium within the first hour, and even after three hours a faint shadow of the dye was still visible.

One cubic centimeter of 35% iodopyracet in rabbit orbits produced prompt miosis of the pupil, and within an hour there was edema of the lids and conjunctiva with definite exophthalmos and, frequently, poor closure of the eyelids. This immediate response receded within the following 12 hours, but histologic study later revealed necrosis of the sclera (Table 1).

Two cubic centimeters of 35% iodopyracet produced marked edema and cellulitis of the orbit, with associated exposure keratitis. Fat necrosis and scleral erosion were striking, and sometimes there was actual rupture through into the eye (Fig. 1).

As hyaluronidase promotes diffusion and rapid absorption of different media in soft tissues, a second series of animal experiments was performed in which the iodopyracet injection was preceded by procaine and hyaluronidase (Wydase). The resulting orbitograms were less satisfactory, but the irritating effects seemed to have been eliminated (Table 2).

Following these animal studies, it was our belief that iodopyracet preceded by hyaluronidase should be safe for orbital injection. At first this combination of drugs was injected behind blind eyes. One cubic centimeter of 3% procaine hydrochloride containing 50 T. R. U. of hyaluronidase was injected into the muscle cone, and the needle was left in place. Twelve minutes later 3 to 5 cc. of 35% iodopyracet was injected, the amount depending upon the orbital resistance. Serial x-rays revealed that most of the dye had disappeared within 10 to 15

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* Leigh, T.: Personal communication to the authors.

† References 2 and 3.

TABLE 1.—*Results of Iodopyracet (Diodrast) Injection in the Rabbit Orbit*

Animal No.	Amount of Iodopyracet, Cc.	Gross Reaction	Time of Autopsy After Iodopyracet	Histopathology
1	0.5	Prompt miosis	4 wk.	Normal eye and orbital tissues
7	1	Miosis; slight edema for 24 hr.	24 hr.	One small perforation with some intraocular hemorrhage; fat necrosis in some areas
12	1	None	48 hr.	One area of sclera acellular; may be the site of partial necrosis
6	1	Miosis; slight lid edema for 24 hr.	2 wk.	One small scar over the site of a perforation into the choroid
10	1	Miosis; slight lid edema for 24 hr.	4 wk.	Normal eye and orbital tissues
8	1.5	Miosis; considerable edema; exophthalmos; two large hemorrhages of retina	2 wk.	A 2 mm. scleral perforation at posterior pole; interior of eye normal
4	2	Miosis; marked edema; exophthalmos; poor lid closure	24 hr.	Considerable fat necrosis; scleral perforations with involvement of intraocular structures; marked orbital cellulitis
2	2	.....	48 hr.	Orbital cellulitis; scleral perforation; thickening of cornea and ring abscess
11	1	None	.....	.....
13	1	Miosis; slight edema	.....	.....
5	1	Slight edema	.....	.....
25	2	Miosis; exophthalmos; poor lid closure	.....	.....
26	2	Miosis; exophthalmos; poor lid closure	.....	.....
27	4	Miosis; exophthalmos; poor lid closure	.....	.....

Fig. 1.—A section from the posterior portion of the eye, showing perforation of the sclera with hemorrhage extending through into the choroid.

minutes. Some patients complained of occipital headache, which developed before the procaine effect had worn off, and most of them suffered an aching of the eye and frontal region for several hours. Considerable edema of the conjunctiva and eyelids developed within 10 or 15 minutes, and this lasted several hours also (Figs. 2 and 3). Histological studies of the eyes which were later enucleated gave no evidence of injury from the irritant—iodopyracet preceded by hyaluronidase (Table 3). At this point the

TABLE 2.—*Effects of Hyaluronidase with Iodopyracet in Rabbits*

Animal No.	Amount of Iodopyracet, Cc.	Gross Reaction	Time of Autopsy After Iodopyracet	Histopathology
14	1	Slight mydriasis; no edema	.....	.....
15	1	Slight mydriasis; no edema	.....	.....
16	1	Slight mydriasis; no edema	.....	.....
17	1	No reaction	1 wk.	Normal tissues
18	1	Slight mydriasis; no edema	.....	.....
24R	1	No reaction	24 hr.	Normal tissues
19	1	No reaction	48 hr.	Normal tissues
20	1	No reaction	.....	.....
21	1	No reaction	24 hr.	A few scattered inflammatory cells
22	1	No reaction	.....	.....
23	1	No reaction	.....	.....
24	2	Slight edema	24 hr.	Normal tissues

Fig. 2.—Case 1, before injection of hyaluronidase (Wydase) and iodopyracet (Diodrast).

Fig. 3.—Case 1, 30 minutes after injection of hyaluronidase (Wydase) and iodopyracet (Diodrast).

TABLE 3.—*Effect of Injection of 50 T.R.U. of Hyaluronidase Followed by Iodopyracet Behind Blind Eyes*

Case No.	Amount of Iodopyracet, Cc.	Gross Reaction	Interval Before Enucleated	Histopathology	Orbitography
1	5	Pain, edema at 3 hr.; E. O. M.* and fundus normal at 10 min., 24 hr.	.....	.....	Satisfactory
2	5	Pain, edema at 3 hr.	20 hr.	No inflammation or necrosis	Satisfactory
3	2	Pain, edema at 2 hr.	14 hr.	No inflammation or necrosis	Unsatisfactory
4	4	Pain, edema at 3 hr.	20 hr.	No inflammation or necrosis	Satisfactory
5	5	Pain, edema at 4 hr.	.....	.....	Unsatisfactory
6	5	Pain, edema at 1 hr.	18 hr.	No inflammation or necrosis	Unsatisfactory
7	5	No pain; slight edema, 1 hr.	20 hr.	No inflammation or necrosis	Unsatisfactory
8	5	Pain, edema at 2 hr.; fundus, E. O. M. unchanged after ½ hr.	.....	.....	Unsatisfactory

* E. O. M. = extraocular muscles.

main problem seemed to lie in the discomfort to the patient after these injections.

Next, orbitograms were made in patients with suspected orbital tumors (Table 4). Two distressing complications were met. Case 4 lost all light perception for approximately 15 minutes in an eye which had previously been found to have 20/20 vision. The immediate fundus picture was that of occlusion of the central retinal artery. The arteries became extremely narrow, and there was segmentation of blood traversing the veins. Vigorous massage was given to

TABLE 4.—*Orbitograms with Suspected Tumors*

Case No.	Condition	Gross Reaction	Orbitography
1	Palpable tumor; routine x-rays negative	No pain; edema 2 hr.	Positive diagnosis, verified at operation
2	Exophthalmos; routine x-rays negative	No pain; edema 2 hr.; vision, E. O. M.,* fundus normal after 10 min. and 24 hr.	Positive diagnosis, not verified by operation
3	Exophthalmos; routine x-rays negative	Pain, edema 12 hr.; vision, E. O. M., fundus normal after 10 min. and 24 hr.	Tumor diagnosed, verified at operation
4	Pulsating eye; hiatus in roof of orbit	Pain, edema 2 hr.; spasm C. R. A.; vision no light perception	Tissues poorly outlined
5	Exophthalmos; routine x-rays negative	Pain, edema 2 hr.; vision, E. O. M., fundus normal at 10 min. and 24 hr.; biopsy at 24 hr. negative	Erroneous diagnosis of tumor
6	Exophthalmos; routine x-rays negative	Pain, edema 4 hr.; vision, E. O. M., fundus normal at 10 min. and 24 hr.	Unsatisfactory
7	Exophthalmos; routine x-rays negative	Pain, edema 4 hr.; vision, E. O. M., fundus normal at 10 min. and 1 wk.	Unsatisfactory
8	Exophthalmos; adherent leucoma	Pain, edema 1 hr.; vision and E. O. M. normal at 10 min. and 24 hr.	Posterior staphyloma demonstrated
9	Exophthalmos; routine x-rays negative	Pain, edema 2 hr.; vision, E. O. M., fundus normal at 10 min. and 24 hr.	Positive diagnosis; verified at operation

* E. O. M. = extraocular muscles.

the eye, but the ophthalmoscopic picture remained the same. Twelve minutes after the injection the vision had returned to counting fingers at 2 ft., and during the following 26 hours it improved to 20/20, at which time the fundus picture was normal except for slight retinal edema.

Case 5 had unilateral exophthalmos and was given an injection of procaine and hyaluronidase, according to the usual routine. Twelve minutes later, 4 cc. of iodopyracet was injected. The orbitograms seemed to demonstrate a definite tumor in the form of a circumscribed area completely devoid of dye. On the basis of this filling defect, a Krönlein operation was performed. However, extensive exploration of the orbit revealed no mass.

Confronted with the false tumor shadow, we performed cadaver injections with Dr. Ted Leigh, who demonstrated that the combination of iodopyracet and hyaluronidase often was not evenly distributed throughout the orbit and frequently false tumors appeared on the films. An incidental finding, which has been mentioned by Gifford,[6] was the gravitation of iodopyracet back into the middle cranial fossa from the orbit. This might have served as a cause for the drowsiness and the occipital headaches which some patients developed.

CONCLUSIONS

Injections of 35% iodopyracet (Diodrast) behind the eye are painful and may produce an alarming amount of tissue necrosis, particularly in the sclera and orbital fat. Such injections should never be performed.

Iodopyracet injection preceded by hyaluronidase is a much safer procedure. However, spasm of the central retinal artery has

been seen as a complication, and therefore this procedure is probably not safe for general use.

Misleading shadows may be outlined when hyaluronidase and iodopyracet are injected. This can lead to an erroneous diagnosis of orbital tumor.

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Therapy of Retrolental Fibroplasia

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Animal experiments of Ashton and others * have made it possible to combine the two apparently opposite points of view concerning the role of oxygen in the pathogenesis of retrolental fibroplasia. The present concept of the etiology of the disease may be summarized as follows: Retrolental fibroplasia is the result of retinal hypoxia. This retinal hypoxia may be induced by two different mechanisms, which may operate singly or in combination:

(*a*) Supplemental oxygen may cause retinal vasoconstriction. Continued vasoconstriction leads to obliteration of the newly formed retinal vessels, and continued supplemental oxygen also leads to suppression of growth of developing vessels. Retinal hypoxia results because of decreased blood supply.

(*b*) Systemic diseases, such as pneumonia (especially interstitial plasma-cell pneumonia) and anemia, may result in retinal hypoxia.

The important role of the choroid in the nutrition of the retina, as emphasized by Ashton, cannot be overestimated in considering the etiology of retrolental fibroplasia.

RETINAL EDEMA IN PREMATURITY

Anatomical investigations † have shown that retinal edema is one of the characteristics of the earliest stage of retrolental fibroplasia. There are also many clinical observations available from which it appears that retinal edema can be one of the first clinical signs of the disease. Michaelson and associates,[5] for example, mentioned "cases of retrolental fibroplasia in which clinically the fundus appearances consist chiefly of a milky opacity of the posterior portion of the fundus with subsequent pallor of the disc. This pallor is of the type seen in vascular occlusion. These cases frequently show no gross clinical evidence of neo-vascularization."

Reese and Stepanik [10] stated:

> There is frequently a paleness or milky appearance to the fundus or the fundus details cannot be seen distinctly. In such instances there seems to be no cloudiness of the media to account for this indistinctness. One gropes for sharpness of detail with various lenses, but never accomplishes it. The cause for this is not clear. We believe it must be due to some intraretinal change during the acute phase and this has lead to some obscuration of the retinal elements.

It seems very likely that the intraretinal change which causes this paleness or milky appearance of the fundus must be retinal edema. The fundus picture of an occlusion of the central retinal artery proves that retinal hypoxia results in retinal edema. As the initial stage of retrolental fibroplasia is due to retinal hypoxia, it seems rather probable that retinal edema can be the main or only symptom of the first stage of the disease.

The repeated and definitely successful result of oxygen therapy in a case of retinal edema in a premature infant, as well as the need, already mentioned in a previous paper [9] of detailed clinical histories, justifies the publication of the following case report.

REPORT OF A CASE

Sept. 2, 1954.—The patient, a boy, was born at home, with a birth weight of 1000 gm. The premature birth had probably been due to maternal pyelitis, with a high fever. The boy was immediately brought to the premature nursery of the Rotterdam Government School for Midwives, where he was placed in an oxygen tent.

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* References 1 to 6.

† References 7 to 9.

During the first month the child was nursed in the oxygen tent, which was kept closed in the usual way (Fig. 1). An oxygen flow of 0.5 liter a minute was administered during the whole month. It appeared from measurements on Nov. 14 that the oxygen concentration within the tent under these conditions was about 65%.

The diet during the first week consisted of feedings, eight times a day, of 25 cc. of maternal milk with 25 mg. of ascorbic acid and 2 drops of a vitamin D preparation. During the first days the child did very well; a marked pallor occurred, however, on the evening of the fifth day, and it looked as though the infant was dying. The condition became better during the night.

Oct. 1.—The first ophthalmological examination revealed no anomalies.

Oct. 2.—The child was removed from the oxygen tent.

Oct. 7.—Ophthalmoscopical examination revealed that the fundus of the right eye had lost its normal red color. The entire fundus had a milky appearance, and the retinal vessels were hardly visible. No hemorrhages, abnormal vascular proliferation, or retinal folds could be seen. The retina of the left eye showed the same milky appearance, but to a less pronounced degree. Parts of the fundus still showed some reddish color. The media of both eyes were clear, and the changes were thought to be due to retinal edema. The child was immediately replaced in the oxygen tent under the same conditions as during the first month.

Oct. 8.—A marked improvement of the ocular backgrounds had occurred. The normal reddish color had reappeared, and no paleness of the retina was visible.

It is impossible, however, to maintain constant oxygen concentrations in an oxygen tent. Moreover, the School for Midwives did not have facilities for measuring oxygen concentrations. Therefore, it was decided to transfer the child to the premature nursery of the Rotterdam Sophia Children's Hospital, where a modern incubator of the Dräger type was available.

Fig. 1.—Oxygen tent closed in the usual way. Oxygen concentration, with an oxygen flow of 0.5 liter a minute, is about 65%.

Oct. 9.—The child was transferred to the Children's Hospital. The weight had increased to 1530 gm. The diet had been changed in the meantime to eight feedings of 35 cc. of maternal milk, with 25 mg. of ascorbic acid and 4 drops of a vitamin D preparation. Ophthalmoscopical examination on the evening of this day revealed that the fundus of the right eye, again, showed a milky appearance; the fundus of the left eye had retained its normal reddish color. The oxygen concentration in the incubator was adjusted at 30%.

Oct. 11.—Both fundi had regained their normal color, with a normal aspect of the retinal vessels. It was decided to lower the oxygen concentration by 2% each day.

Examination of the blood revealed a hemoglobin content of 10.4 gm. per 100 cc.; a red cell count of 2,800,000, and a white cell count of 9700, with 48% lymphocytes, 28% polymorphonuclear cells, 5% band forms, 4% eosinophiles, 1% basophiles, and 14% monocytes. From this day on the child received 3 drops of syrup of ferrous chloride a day.

Oct. 12.—A count of the reticulocytes revealed 74%.

Oct. 13.—Both eyes showed a normal fundus. The oxygen concentration within the incubator was reduced from this day at a rate of 1% a day.

Oct. 16.—Ophthalmoscopical examination again revealed no anomalies in either eye.

Oct. 19.—As the eyes were normal again, it was decided to discontinue oxygen administration.

Oct. 20.—The diet was changed to 40 cc. of maternal milk eight times a day.

Oct. 23.—The weight had increased to 1860 gm. The eyes still showed no anomalies. The child was brought back to the premature nursery of the School for Midwives. The general condition of the child had remained excellent during the entire stay in the Children's Hospital.

Oct. 28.—No supplementary oxygen had been given since the child's readmission to the School for Midwives. Ophthalmoscopical examination again revealed a marked retinal edema in both eyes, without retinal hemorrhages or other anomalies. Again, a flow of 0.5 liter of oxygen a minute was administered to the oxygen tent. This time the tent was not closed in the usual manner. Its open side was closed with a coverlet, and the oxygen flow was maintained through a tube which was led beneath the coverlet. The end of the tube was placed at a distance of 5 cm. from the child's face.

Oct. 30.—The condition of the eyes had not improved. Therefore, the end of the tube was placed at a distance of 3 cm. from the child's face.

Nov. 1.—A marked improvement in the ocular backgrounds had occurred. Some edema, however, was still present. Oxygen administration was continued in the same way.

Nov. 4.—No further improvement had occurred. Oxygen therapy was continued.

Nov. 8.—The whole retina of the right eye and the larger part of the retina of the left eye again showed a milky appearance. The macular regions were visible as typical red spots surrounded by retinal edema such as are encountered in cases of occlusion of the central retinal artery. No retinal hemorrhages or folds were present. The flow of oxygen was increased to 1 liter a minute.

Nov. 10.—The condition of both eyes had markedly improved. The retinal edema had disappeared largely in both eyes. The oxygen flow was again reduced to 0.5 liter a minute.

Nov. 13.—No more retinal edema was present. Examination of the blood revealed a hemoglobin content of 55%; the red cell count was 2,290,000. The child weighed 2450 gm. From this day 150 mg. of reduced iron a day was given.

Nov. 14.—No ocular anomalies could be found. We felt, however, rather unhappy that we could not determine the oxygen concentration in the oxygen tent. On our request, the chief pediatrician of the Sophia Children's Hospital measured the oxygen concentrations in the oxygen tent with the apparatus belonging to the Dräger incubator. All measurements were performed with an oxygen flow of 0.5 liter a minute. The following concentrations were found:

(*a*) Oxygen tent normally closed: 67% and 64%

(*b*) Oxygen tent closed with a coverlet: 48% and 39%

(*c*) Oxygen tent without coverlet: 28% and 26%

(*d*) Oxygen concentration within the premature nursery: 25%

It was decided to continue the oxygen administration with 0.5 liter a minute without any cover over the open side of the tent.

Nov. 16.—No ocular anomalies were visible.

Nov. 21.—Some retinal edema had recurred in both eyes.

Nov. 24.—The retinal edema had increased in both eyes. The open side of the oxygen tent was again closed with a coverlet.

Nov. 26.—The retinal edema had almost totally disappeared. Measurements of the oxygen concentration in the tent were again performed. With an oxygen flow of 0.5 liter a minute the results were as follows:

(*a*) Oxygen tent closed with a coverlet: 38.5% and 38.5%

(*b*) Oxygen tent without coverlet: 27% and 27%

The tent was kept closed with a coverlet for another day.

Nov. 27.—The retinal edema had totally disappeared. The coverlet was replaced by a double layer of gauze bandage. The hemoglobin content of the blood was 65%; the red cell count, 2,860,000.

Nov. 28.—The eyes showed a normal fundus. Measurements of the oxygen concentration within the tent were again performed, with an oxygen flow of 0.5 liter a minute. The following concentrations were found:

(*a*) Oxygen tent covered with a double gauze layer: 38% and 38%

(*b*) Oxygen tent covered with a single gauze layer: 35% and 35%

The double layer of gauze was replaced by a single layer (Fig. 2).

Nov. 30.—No ocular anomalies were found. The weight of the child had increased to 3040 gm. From this day, only the upper half of the open side of the oxygen tent was covered, and by a single layer of gauze.

Dec. 6.—No retinal anomalies were present.

Dec. 13.—The fundus of both eyes was normal again. The weight of the child had increased to 3520 gm. From this day the oxygen tent was kept open. The oxygen flow of 0.5 liter a minute was continued.

Fig. 2.—Open oxygen tent, covered with a single gauze layer. Oxygen concentration, with an oxygen flow of 0.5 liter a minute, is about 35%.

Dec. 20.—Again, no ocular anomalies were found. The hemoglobin content of the blood was 70%; the red blood cell count was 3,510,000. Administration of oxygen was discontinued.

Dec. 28.—The eyes were normal. The weight had increased to 3960 gm. The child was discharged from the nursery.

Further Course.—Regular examination of the child up to May 20, 1955, revealed normal eyes in a boy who was a picture of health.

COMMENT

In a symposium on retrolental fibroplasia held in 1954 at the annual meeting of the American Academy of Ophthalmology and Otolaryngology,[11] a dramatic negative statement on the therapy during the active phase of the disease was given in the chapter on "Management." The only recommended therapeutic measure in this stage was the use of mydriatics to avoid posterior synechias. Moreover, the conclusions of the panel were as follows: "We feel that there is insufficient evidence available to support the idea that R. L. F. is benefited by oxygen therapy." The panel expressed its fear of oxygen even still more emphatically by preferring rapid transfer to normal air to gradual weaning of the child from oxygen.

This negative statement does not accord with the experience of various clinical students of the disease. The careful clinical observations, and the promising therapeutic results of Szewczyk,[12] published in 1952, appear to have been neglected, as well as the affirmation of these results by many other clinical investigators. Dramatic improvement when babies with ophthalmoscopic changes of short duration were treated with oxygen have been described by Swart-van der

Hoeven and Mak,[13] Jefferson,[14] Huggert,[15] and Bedrossian and associates.[16] The last author even described a case in which oxygen therapy was given an infant three times, with successful results, because of recurrence after each withdrawal of oxygen. Later, Zanén and associates,[17] and Bourgonnier and associates,[18] concluded that administration of oxygen could arrest the disease and that this therapy also should be terminated gradually. Ashton [3] recently concluded from his animal experiments "that it may be dangerous to return cases of retrolental fibroplasia to oxygen incubators." He added, however: "But this point needs further investigation, especially in view of the contrary clinical experience of Szewczyk (1953)." Moreover, it is not clear from Ashton's paper whether the kittens in his experiments were gradually withdrawn from oxygen. In the same paper he also stresses the differences between the eye of full-term kittens and that of premature infants.

Still more recently, Bedrossian and associates [19] published new data on the effect of oxygen therapy in retrolental fibroplasia. Seven infants were given oxygen therapy because their retinopathy appeared progressively worse on repeated examinations. All these infants had a rapid and definite improvement in their fundus when oxygen administration was reinstituted. These authors concluded that a gradual reduction in the oxygen concentration of the infant's environment should be made before oxygen is stopped completely, and that oxygen therapy is of value after retinopathy develops if judiciously used and gradually withdrawn.

Retinopathy of prematurity, in its initial phase, as well as in the more advanced stages, must be considered an imminent danger to the visual faculty of the child. The pediatrician in charge of a premature nursery should therefore urge the consulting ophthalmologist to prescribe concrete therapeutic measures to prevent the development of blindness. Despite the negative conclusions of the above-mentioned 1954 symposium, the ophthalmologist will seem quite justified in giving the child a fair chance for improvement by readministering oxygen. A concentration of 40% oxygen probably will be sufficient to reestablish a normal-looking retina. This oxygen concentration is to be reduced very gradually. Careful control of the eyes is necessary, and the flow of oxygen must be increased again when the retinal changes reappear. It will often be necessary to continue the ophthalmoscopic controlled oxygen therapy until the infant has reached a weight of about 3000 gm.

The bad consequences of sitting with folded hands are clearly shown in the recent study by Krause [20] on the effect of retrolental fibroplasia. It appears from this study that in 91 of 107 children the disease resulted in a vision of less than 10/100 in the best eye.

The case report presented is intended as another plea for active therapy, consisting of readministration of oxygen in all cases in which the initial stages of retrolental fibroplasia are diagnosed. Other therapeutic measures should be directed against anemic symptoms, which are present in most premature infants.

SUMMARY AND CONCLUSIONS

Retinal edema in prematurity is one of the most important warnings of the presence of retinal hypoxia. It may be the first or single symptom of retinopathy of prematurity and has, therefore, to be considered as an imminent danger to the visual faculty of the child.

A case of retinal edema in a premature infant with a birth weight of 1000 gm. is reported. The child had been kept in an oxygen concentration of about 65% during its first month of life. No ocular anomalies were present at the end of this month. Retinal edema developed within one week after withdrawal of the oxygen supply. The child was treated with oxygen during a period of more than two months after its first withdrawal from the oxygen tent. In these months the edema reappeared four times after the oxygen concentration had been reduced, and disappeared every time after reinstitution of the oxygen treatment, at a higher level. A definite cure was established

after the child had reached a weight of 3000 gm.

It is concluded that the consulting ophthalmologist of a premature nursery should advise active treatment with ophthalmoscopically controlled, careful administration of oxygen in all cases of retinal edema or other initial symptoms of retrolental fibroplasia in premature infants.

The pediatrician in charge of the premature nursery of the Rotterdam Government School for Midwives is Dr. J. T. Swart-van der Hoeven. Dr. H. Reerink, Director of the Sophia Children's Hospital, cooperated by measuring repeatedly the oxygen concentration in the oxygen tent of the School for Midwives.

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Case Reports

MILIARY BLASTOMYCOSIS WITH METASTATIC SPREAD TO POSTERIOR UVEA OF BOTH EYES

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Blastomycosis, owing to its relative infrequency, has received little comment in medical literature, particularly with relation to the eye. In 1946, Cassady [1] reported a case of ocular blastomycosis metastatic to the iris. The presentation included a complete review of the literature.

The following case of metastatic blastomycosis is of interest, since the blastomycotic lesions which were observed and recorded clinically could be substantiated by means of histopathological studies.

REPORT OF CASE

W. F., a 39-year-old Negro, was admitted to Duke Hospital on Dec. 26, 1953, because of severe fever, chills, weakness, and generalized pustular eruption, of two weeks' duration. He had been treated with systemic sulfadiazine, oxytetracycline (Terramycin), and penicillin in the usual doses, without effect. The patient's past history revealed a gradual weight loss of 20 to 25 lb. and anorexia during the previous year.

On examination the patient was found to have a temperature of 39 C. Pustular and papular skin lesions covered the face, arms, and trunk (Fig. 1). One white spot would be seen along the superior temporal artery of the right fundus. There was generalized suppression of breath sounds, accompanied by occasional basilar rales. X-rays of the chest showed a diffuse, miliary-like infiltration throughout both lungs, more marked in the midportions, with possibly the extreme apices clear or only slightly involved. Laboratory tests were as follows: Blood: hemoglobin, 9.5 gm. per 100 cc.; W. B. C., 25,500; polymorphonuclear leucocytes, 90%; stab forms, 2%; small lymphocytes, 4%; monocytes, 4%. Urinalysis: specific gravity, 1.017; protein, trace; sugar, 0; W. B. C. 6-10/h.p.f.; occasional R. B. C.; fine granular and hyaline casts. Stool: negative. Serological tests for syphilis: negative. Sedimentation rate: 59 mm. in one hour, uncorrected. Skin tests: old tuberculin, negative in dilutions of 1,000,000 to 1,000; blastomycin, negative; coccidioidin, negative; histoplasmin, negative. Blood chemistry: nonprotein nitrogen, bilirubin, and total protein, normal. Spinal fluid studies: negative. Smears for acid-fast bacilli: pustular skin lesions, negative; bone marrow, negative. Blood culture: negative. Gastric washings: negative.

Fig. 1.—Postmortem photograph, showing pustular lesions covering eyelids and face.

The tentative diagnosis was miliary tuberculosis, with fungous infection to be excluded.

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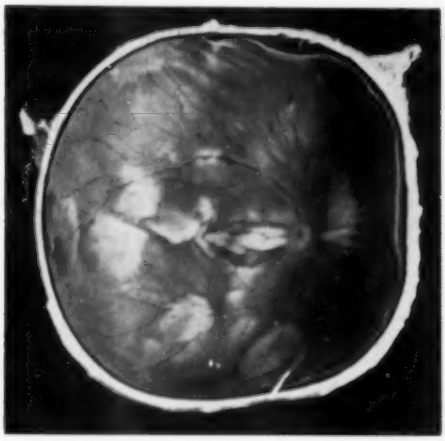


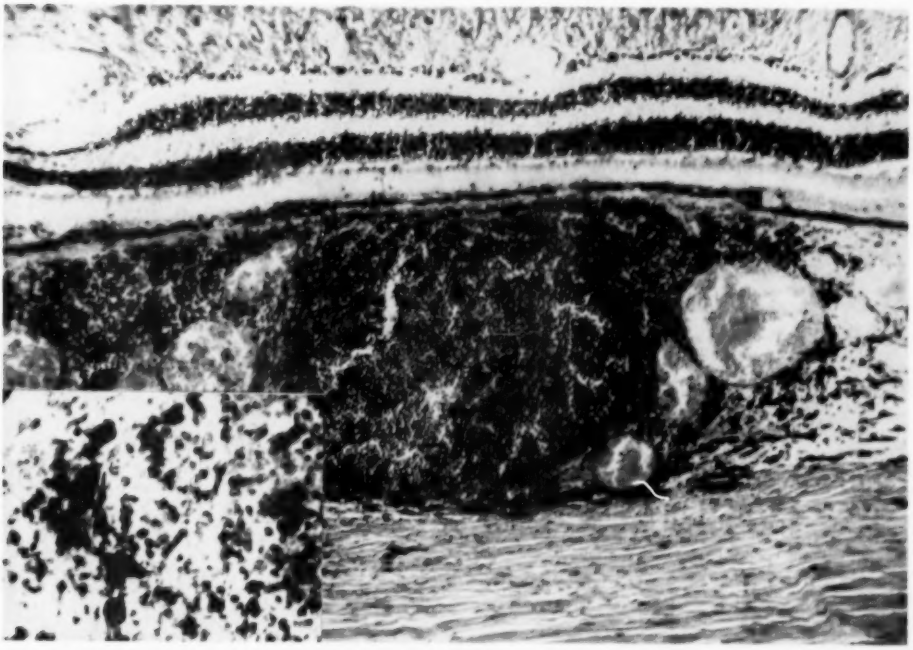
Fig. 2.—Large granulomatous lesion in choroid, pushing retina forward.

The patient's course in the hospital was rapidly downhill and was characterized by high fever, disorientation, nausea, vomiting, and abdominal cramping. Daily therapy with isoniazid, 300 mg., and a preparation of streptomycin sulfate and crystalline dihydrostreptomycin sulfate, equal parts (Distrycin), 1 gm., was instituted immediately. Two whole-blood transfusions, of 500 cc. each, were given. In order to evaluate the lesion in the right fundus, an ophthalmologic consultation was requested. Because of the patient's poor condition and disorientation, the examination was necessarily limited.

The lids were covered with pustular lesions, in various stages of development. The conjunctivae and corneae were normal. The pupils were round and regular and responded well to light. Through the dilated pupils, the media appeared clear. The fundus lesions, which were usually found near an artery, consisted of a number of round, yellowish-white areas, O.U., approximately one-eighth the size of the disc. The lesions seemed to be in different stages of development, some showing atrophy of the pigment layer of the retina immediately surrounding the lesion. One white, fluffy, exudate-like lesion was seen along the superior temporal artery about 3 D. D. from the disc in the right eye.

Repeated smears of the pustular skin lesions finally revealed the Blastomyces organisms, on Jan. 7, 1954. The patient was immediately started on stilbamidine isethionate, 150 mg. in 300 cc. of 5% dextrose. This therapy was ineffectual, and 24 hours later the patient died.

Fig. 3.—Low-power magnification of granulomatous inflammatory type of lesion in choroid. Inset: high-power magnification of double-contoured budding form with highly refractile wall.



Autopsy was limited to examination of the eyes. On gross section, light grayish-yellow areas, 1 to 3 mm. in diameter, were seen along the course of the vessels of the left eye. On the cut edge of the section, large, granulomatous-like lesions in the choroid pushed the retina forward (Fig. 2). Microscopic examination showed that the lesions were limited to the choroid of the posterior half and were mainly concentrated in the posterior third of the eye. Some of the lesions were of the granulomatous inflammatory type, while others were typical of purulent suppurative inflammation. An occasional granuloma, composed almost entirely of epithelioid giant cells, was present. Most of the lesions showed a central area of necrosis or suppuration, containing large numbers of polymorphonuclear leucocytes with scattered macrophages and giant cells in the periphery. Numerous yeast-like organisms were present. All of these were quite large and had a double-contoured, highly refractile wall, accompanied by occasional budding forms (Fig. 3). The microscopic picture of the right eye was similar to that of the left.

The final diagnosis was miliary blastomycosis with metastatic spread to the posterior uvea of the eyes.

David S. Johnson, M.D., Department of Pathology, Duke University School of Medicine, furnished the photomicrographs.

REFERENCE

1. Cassady, J. V.: Uveal Blastomycosis, Arch. Ophth. **35**:84, 1946.

Annual Reviews

PHYSIOLOGICAL CHEMISTRY OF THE EYE

A Review of Papers Published During 1954

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The present review is concerned principally with reports published during 1954 on the physiological chemistry of the eye. As most of the pertinent papers presented at the XVII International Congress of Ophthalmology are available only in abstract form, any mention of them is necessarily brief, as are references to articles from the Japanese literature, which could be judged merely on the basis of short résumés; such references are indicated by an asterisk. Subdivisions of the material follow that of previous years, and, again, several papers on related subjects have been added to make the review more comprehensive.

CORNEA

Woodin has shown in the past that the acid mucopolysaccharide of cattle cornea is combined with protein. In a recent study,[1] the author examined the phenol-insoluble mucoid of this tissue. The content of tyrosine, tryptophan, and hydroxyproline and the reaction with collagenase seem to indicate that the protein fraction is not collagen or a collagen derivative, in contrast to previously expressed opinions. On the basis of the results of electrophoretic and ultracentrifugal studies on the material, Woodin considers the protein-mucopolysaccharide linkage to consist of an ion pair bond between sulfuric ester groups and the amidine group of arginine. Similarities between whole rat cornea and sclera and a system composed of gelatin from pig skin and mucoitin sulfuric acid (acid mucoid of the cornea) are inferred by Loeven and von Walbeck[2] on the basis of the influence of pH changes on swelling properties and the degree of turbidity at a physiological water content. They concluded that corneal and scleral connective tissues are related to complex colloid systems, as defined by Bungenberg de Jong, and that collagen and mucopolysaccharide are the two essential constituents. Meyer *[3] summarized some aspects of the chemistry of the ground substance in connective tissue. Up to the present the mucopolysaccharides, hyaluronic acid, chondroitin sulfates A, B, and C, chondroitin, and keratosulfate have been isolated. From the cornea of cattle, chondroitin sulfate A and keratosulfate were obtained. The characteristics of these components were described in the study reviewed last year.[4]

In an attempt to discover the mechanism operative in preserving normal hydration of the cornea, Davson[5] carried out experiments on excised rabbit eyes and, by procedures different from those used previously for this purpose, demonstrated the importance of a normal-functioning metabolism of the epithelium and endothelium for the steady state. He examined hydration of the cornea at 7 and 31 C respectively and observed that at the lower temperature hydration is great, due to the suppression of metabolism, and that this effect can be reversed by raising the temperature to 31 C. Anoxia produced the same effect. The hydration appears to be independent of the rate of evaporation from the eye surface. The active transport mechanism thus shown may consist of active removal of water by the epithelial layer, favoring the passive diffusion of crystalloids from the stroma, or the active extrusion of crystalloids, resulting in a passive removal of water. Potts, Goodman, and Johnson[6] investigated

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the effect of chemical and physical damage to the rabbit corneal epithelium and endothelium on the transfer rate of Na^{22} from the cornea to the aqueous humor and to artificial tears in an applicator. After the radioactive indicator was injected intracorneally, its concentration decreased rapidly in the tissue and reached a maximum in the aqueous humor within 20 minutes. This behavior of radiosodium was not altered by injury to the endothelium despite clouding and swelling of the stroma, seeming to indicate that sodium movement and corneal dehydration are not strictly connected phenomena. Results of experiments with damaged epithelium suggest that this cell layer is relatively impermeable to the sodium ion. As a possible explanation of their observations, the authors offer the hypothesis that sodium is actively secreted from the cornea into the aqueous humor without participating essentially in the dehydration of the cornea. At the International Congress, Potts [*7] commented upon his isotope experiments on corneal permeability. He felt that the results indicate that ion transport properties are located in the corneal endothelium. The importance of the metabolic activity of epithelium and endothelium for corneal hydration was also studied by Pau [8] in an extensive investigation. Excised beef corneae were immersed in Ringer's solution, and the weight gains due to absorption of fluid seemed to be related to the intact condition of the epithelium and, to a slighter degree, of the endothelium. Interference with normal metabolic processes of the cell layers resulted in excessive weight increase in the corneae. Bowman's and Descemet's membranes played a minor part in the protection of the stroma against excessive uptake of fluid. A dehydrogenase system was demonstrated histochemically in the deep and middle layers of the epithelium and in the endothelium in sections of the cornea, and a cytochrome-cytochrome oxidase system was shown to be located predominantly in the nuclei of the endothelial cells and the cells of the basal and superficial epithelial layers. Pau suggests that the distribution of these enzymes influences the site of the deposition of metals in corneae under pathological conditions. Smelser and Chen [*9] compared structure and hydration properties of corneae of the carp and the guinea pig. They report that the epithelium of the latter is thinner and its stroma gives a stronger periodic acid-Schiff reaction than those of the fish. Descemet's membrane and endothelium appear to be absent in the carp cornea. The swelling tendency of the corneal connective tissue in the carp is less marked than in the guinea pig. In both species the epithelial coverings are essential for the maintenance of normal hydration. The guinea pig endothelium is of relatively greater importance in this respect than that of the fish, but the reverse is true of the epithelium. Interestingly, the swelling power of the guinea pig cornea is much more dependent on salt content than is that of the carp. The results of this informative study on corneae which are adapted to air or to aquatic environment suggest that the freshwater fish cornea is able to resist hydration by virtue of the difference in polymerization of mucoid, the difference in importance of electrolyte-controlled swelling, and the thickness of its epithelium. Langham,[10] in a study on glycolysis in the rabbit cornea, observed that the epithelium produced lactic acid at an even higher rate in vivo than in the excised cornea in the presence of oxygen. He explains this tentatively as being due to injury of the epithelium after excision. The rate of acid production in the stroma was found to be unaffected by oxygen tension, in contrast to the marked decrease in acid production in the epithelium under such conditions. The rate of accumulation of the acid in the whole excised cornea decreased 74% under aerobic conditions. The aerobic production of lactic acid in the rabbit cornea may indicate a species peculiarity, since it was not observed by de Roetth [11] in his study on the cattle cornea. Langham noted further that lactic acid accumulated in the cornea of the living rabbit exceeds that in the excised cornea under aerobic conditions. Possible explanations of this surprising fact are offered.

In a short series of experiments, Giardini and Gandolfi [12] used Maurice and Giardini's instruments to measure the corneal thickness of rabbit eyes and determined corneal hydration following thermal burns at the limbal area. Within 48 hours the center of the cornea exhibited a decrease in its thickness, which had reached a maximum 8 hours after the trauma. This phase was followed by an increment of 50% above the normal after 48 hours. Osmotic forces and metabolic activity of the epithelium and endothelium are thought to provoke the dehydration of the central part of the cornea. The increase of corneal thickness in the second phase may be caused by injury to the endothelium. Michaelson, Herz, and Kertesz [13] conducted a study to determine the effect of increased oxygen concentration on vessel budding in the adult rabbit cornea. New growth was induced by standard thermal burns at the limbus. A comparison of the extent of vessel growth was made between animals incubated for six days in an atmosphere containing 50% oxygen and animals in air. No effect on growth was produced by the higher oxygen concentration. The significant participation of the active glycolytic and respiratory metabolism of the corneal epithelium and endothelium in maintenance of corneal transparency was stressed again by Langham. In a review paper,*[14] he discussed in detail the relation of corneal respiration to hydration and turbidity in the light of recent developments in this field. Cogan and Kinsey *[15] took issue with the criticisms of the deturgescence theory of corneal transparency, according to which maintenance of this state depends on osmotic forces acting at the corneal surface, with epithelium and endothelium functioning as semipermeable membranes. Smelser, in the discussion, attempted to reconcile the two theories, in which either metabolic activity of various structures or physical forces are seen as predominantly effective mechanisms. At the International Congress, Woodin *[16] discussed the mucopolysaccharide-protein interaction in the cornea and mentioned the importance of the polysaccharide components of this tissue with reference to their contribution to the swelling pressure of tissues and to the problem of corneal hydration. In an excellent study, Maurice *[17] reviewed critically the present concepts of transparency of the cornea and loss of it due to swelling and presented physical aspects of the problem. Components of scattered light from individual fibers appear to be destroyed because of the orderly arrangement of the elements by mutual interference in any direction but that of the incident light. On the other hand, upon mechanical deformation (during swelling), components of the light scattered by separate fibers may reinforce each other.

Teng and Katzin have shown previously (1953)[18] that the basement membrane of the corneal epithelium can be distinguished from Bowman's membrane by the periodic acid-fuchsin stain. This can be demonstrated particularly well in cattle cornea. Such material and human eyes were used by LaTessa, Teng, and Katzin [19] to identify in this structure—by histochemical methods—lipids, free aldehydes and ketones, acetalphosphatides (plasmalogen), glycogen, other carbohydrates, and reticulum fibers. Corneae injected intralamellarly with trypsin, chymotrypsin, ribonuclease, hyaluronidase, or ptyalin were incubated, sectioned, and stained according to the Schiff technique. From these and other experiments, the investigators concluded that the basement membrane of the corneal epithelium contains reticular fibers, polysaccharide complexes, and the phospholipid plasmalogen. Lipid solvents seemed to effect a loss of cohesion between the epithelium and the membrane, suggesting that a lipoid layer helps to maintain adherence of the epithelium to the basement membrane. Plasmalogen may play a part in this phenomenon. Alagna [20] observed positive Hotchkiss reactions and metachromatic staining with toluidine blue in the epithelium, endothelium, Bowman membrane, and Descemet membrane and in the peripheral zone of the stroma of the rabbit and rat corneae. Maltase inhibited the Hotchkiss stainability only in the epithelium. Quantities and localization of the corneal mucoid were similar in adult and

10- to 20-day-old animals. Hotchkiss-positive granules were less numerous in the epithelium of young animals. There is no disagreement between these results and those reported by Aurell and Holmgren,[21] but the author did not extend the investigations to fetal corneae. Krwaricz, Seidler-Dymitrowska, and Vorbrodt *[22] determined the ascorbic acid content in corneal stroma cells in investigating their resemblance to active mesenchyme. Corneae preserved for 14 days in liquid petrolatum U. S. P. were used for the histochemical demonstration of the reduced form of ascorbic acid. The cells accumulated ascorbic acid within four to six days of preservation under anaerobic conditions. During this period, the cells changed their shape, threw out processes, and probably became mobile, like cells of active meshenchyme. The period of stimulation was followed by rapid destruction and necrosis. At last year's International Congress, François and Rabaey *[23] reported attempts to quantitate interreactions between basic stain and mucoids in histological preparations. The publication of the full details of this investigation is awaited with interest.

Cogan and Kuwabara * reported a most interesting study of lipogenesis by cells of the cornea. Experiments by Simms and associates on fat formation in chick fibroblasts had led to the conclusion that serum contains lipoid-soluble substances which can be converted into fat. In the present work, serum was found essential to lipogenesis. It could not be replaced by a dextrose-saline or a glycerin-saline medium. Healthy cells of the rabbit corneal epithelium, stroma, and endothelium produced fat by a dynamic process when incubated with oleic acid or sodium oleate in the presence of serum. Other fatty acids failed to give positive results. Hydrolyzed rabbit fat or hydrolyzed olive oil induced lipogenesis under the conditions of the experiment. Obviously, this study is of importance for the basic problem of neutral-fat formation by nonadipose tissue.

* References *24 and 25.

Jakus [26] investigated and beautifully documented the electron microscopic structure of the rat cornea. Among the many observations, those describing interdigitating processes of epithelial cells and filaments in their cytoplasm are especially interesting. The fibrous nature of the basement membrane which she observed may have relation to the histochemically demonstrated fibrils in the study of LaTessa and co-workers. According to Jakus, the Descemet membrane of the rat appeared essentially "structureless," in contrast to the fibrillary pattern discernible in this membrane in cattle, which she recently described.[27] François, Rabaey, and Vandermeerssche [28] examined the ultramicroscopic structure of cornea and sclera from human and cattle eyes and arrived at results similar to those of Schwarz.[29] Scleral fibers varied in thickness from 400 to 3,300 A. and were arranged irregularly. The fine corneal fibers have diameters of 300 to 350 A. and are embedded in an amorphous mucoid medium—the cement substance of Schwarz. Interesting is the observation of the authors that diameter and periodicity of the corneal fibrils remain unchanged after swelling of the cornea in distilled water. The effects of treatment with hyaluronidase are interpreted as indicating that the mucoid is probably not eliminated, but is covered by some substance which makes these coats indistinct. It may be of relevance that electron-microscopic examination of the normal human eardrum membrane by Marotzke [30] also showed that the cement substance surrounding the fibrils of this tissue is not markedly influenced by the action of hyaluronidase. Epithelium, stroma, and Bowman and Descemet membranes of human and rabbit corneae were studied by Rouiller, Danon, and Ryter [31] with the phase-contrast and the electron microscope. Their report does not contain any new observations, and the value of the paper rests in the comparison of results obtained with various techniques.

Thompson and Harrison [32] investigated factors which affect antibody production in the rabbit cornea. Corneal juice and sera of

animals which had earlier received protein antigens by intracorneal injection were titrated for antibody, using Boyden's hemagglutination technique. At later periods, corneal antibody titers consistently surpassed those of the sera or were positive when sera were negative. Topical administration of cortisone reduced the corneal reaction and antibody formation. Local β- or x-radiations did not change these processes, but total body irradiation with shielding of the head nullified corneal reaction and antibody formation. The latter observation is of considerable radiobiological interest. Subconjunctival injection of an 8% solution of cysteine exerted, according to McDonald,[33] a protective action in all corneal structures against ionizing radiation. The injection of the amino acid preceded by one hour the exposure of the rabbit eye to 40,000 rep of beta-radiation delivered by an Sr^{90} applicator. Blum *[34] used a chemical-colorimetric method to measure the concentration of ioniazid (isonicotinylhydrazide) in the aqueous humor of the rabbit after corneal iontophoresis with local anesthesia. The drug levels were found to be considerably higher than is necessary for bacteriostasis.

AQUEOUS HUMOR

Wunderly and Cagianut[35] used paper chromatographic techniques in an effort to separate and identify free amino acids in the aqueous humor of normal and diseased human eyes. Most of the amino acids which are present in the plasma could scarcely be detected in the aqueous humor with the sensitive technique employed. The aliphatic amino acids of low molecular weight predominated, and free alanine was abundant. Plasmoid aqueous humor showed an increase of free amino acid concentration. In acute infections, the basic amino acids were predominant. Aqueous humor from patients with mature cataract contained free methionine in considerable quantities. Wunderly, Steiger, and Böhringer[36] attempted to determine the proteins in aqueous humor of individual human eyes by a micromethod, with a standard error of $\pm 3.7\%$. For paper electrophoresis, two or three samples were pooled and concentrated. Alpha-1 and α-2 globulins could not be distinguished from each other, and the standard error for determination of albumin is given as $\pm 3.2\%$. The mean protein content was 14.9 mg. per 100 cc., of which 63% was albumin, 9% α-globulin, 10.5% β-globulin, and 17.5% γ-globulin. Esser, Heinzer, and Pau[37] pooled and concentrated human aqueous humor from 11 normal eyes for paper electrophoresis analysis of the proteins and for comparison with samples of cerebrospinal fluid. The patterns obtained from these two fluids were similar. In material withdrawn 14 to 24 hours post mortem, the γ-globulin appeared to be increased. As shown previously by other investigators who used aqueous humor from rabbit and cattle, there is present a component which has greater mobility than albumin. Green and Leopold[38] investigated the presence of corticosteroids in the aqueous humor of normal rabbits by various absorption spectrometric methods. Evaporation residues of chloroform extracts of aqueous humor were subjected to the blue tetrazolium test. With this technique, the concentration of the corticosteroids was estimated to be 2 to 4 γ/ml. Repeated paracentesis heightened the level of the α-ketol group, as detected selectively by this method. To provide confirmation for the interpretation of the absorption spectra, particularly the absence of the Δ^4-3-keto group, and the presence of the 11-OH and 17-OH groups in the aqueous humor, the authors plan to use paper chromatography.

In order to estimate the rate of flow of aqueous humor, Bárány and Wirth[39] infused rabbits with paraaminohippuric acid intravenously for five to six hours and obtained a relatively constant plasma level. Repeated blood samples were taken during the infusion. The aqueous humor of one eye was withdrawn before the infusion was stopped, and that of the other eye was removed one hour later. From the concentration of the test substance in the various samples, the steady-state distribution ratio of aqueous to plasma was estimated. The results, in 12 out of

13 animals, were in agreement and showed an average K_{out} of about 0.01. Figures obtained from the right and the left eye were of the same order. In other groups of animals, the flow seemed to have come to a stop after puncture of the first eye, and in still others abnormal values and marked differences between the two eyes were observed. Several sources of this error are considered. Kinsey and Palm *[40] measured the rates of entrance of two test substances, Na^+ and SCN^-, into the anterior and posterior chambers of the rabbit, and they reviewed this work at the International Congress. Differences in these rates supported the previous theory that Na^+ enters the posterior chamber by an active process, whereas SCN^- enters by diffusion. Davson *[41] followed the passage of thiourea from the plasma to the aqueous humor and the lens and its distribution in the lens at an early stage of equilibration. The anterior half of the lens contained about twice as much thiourea as the posterior half, and the pupillary size did not influence the results. It seems, then, that the test substance entered the lens mainly from the aqueous humor through the posterior chamber. Lenses of young animals (rabbits) took up plasma thiourea more rapidly, because of an accelerated net flow of aqueous humor, better penetrability of the lens substance at this age, or a "more favorable area-volume relationship" between lens surface and posterior chamber. Friedenwald *[42] reviewed and expertly discussed the ascorbic acid turnover in the aqueous humor. The difference in ascorbic acid concentrations in the aqueous humor of the posterior and the anterior chamber was shown to be caused by outdiffusion from the anterior chamber. The results of previous studies by the author, Kinsey, Linnér, Becker, and others are presented to give a rounded picture of the mechanism involved in secretion of the compound and its influence on aqueous flow. The principles of aqueous flow determinations based on ascorbic acid transfer across the blood-aqueous barrier were reviewed by Langham.*[43] He also discussed the techniques which have been used for such investigations in the rabbit and in man. Davson,[44] in a broad discussion of the role of active transport by specialized cells of complex organs in the production of certain body fluids, refers to the many studies conducted in the past on the origin of aqueous humor and also to the changes in concept from secretion to ultrafiltration to active transfer and the combination of these mechanisms.

Several articles by Bárány † (one in conjunction with Scotchbrook [47]) deal with in vitro studies in which the resistance of flow through the angle of the anterior chamber was tested under the influence of testicular hyaluronidase. On the basis of Persson's findings of metachromasia in the trabecular meshwork of human eyes and its sensitivity to purified hyaluronidase, Bárány devised several techniques to show the presence of a mucopolysaccharide barrier to outflow of aqueous humor. The first series of experiments, conducted on cattle eyes, indicated that there is such a barrier, since after treatment with hyaluronidase the resistance of the filtering eye dropped to about one-half the initial value. The resistance that remained after the action of hyaluronidase was considered to be due to the fibroblastic nature of the chamber angle. This basic resistance is enhanced by "water-proofing" with ground substance containing hyaluronic acid. To influence the resistance of the barrier by changing the balance between production of ground substance by the fibroblasts of the trabeculum and its destruction by the allegedly always-present enzyme in the aqueous humor, several ingenious techniques were elaborated, which cannot be described here; but the results will be mentioned in view of their potential practical applicability. Of a series of enzyme inhibitors, only iodoacetate caused a rapid fall of the resistance of the barrier. On the other hand, dilution of the aqueous humor with an approximately equal amount of buffer also caused a drop in resistance; this drop was large if the aqueous was wholly replaced by buffer. Whether the unphysiological conditions thus induced or the me-

† Bárány,*[45] Bárány and Wirth.[39]

chanics of the necessary washing procedure caused the breakdown of the barrier is not definitely known, but experiments with repeated interruptions of the perfusion, drawing back and gently blowing in aqueous humor, showed that such mechanical trauma damages the barrier. In vivo experiments by Bárány did not support the hypothesis of the importance of a hyaluronidase-sensitive barrier for the regulation of the intraocular pressure. According to the reports of other investigators, the hyaluronidase inhibitor trigentisic acid did not affect the intraocular pressure, nor did the injection of hyaluronidase into the anterior chamber or vitreous or under the conjunctiva alter significantly the intraocular tension.

The question as to why ascorbic acid concentration is maintained in the aqueous humor at a level exceeding that in the blood was investigated by Purcell, Lerner, and Kinsey [48] on patients with and without cataract and on aphakic eyes. Individual variations in the ascorbic acid content of the aqueous humor and serum were considerable. The lower value in the aqueous humor obtained from eyes with cataract corresponded to a similarly low level in the blood; the concentration in the aqueous humor changed with the serum concentration in both groups of patients. Thus, synthesis of ascorbic acid or reduction of dehydroascorbic acid by the normal lens appears improbable. This contention is further supported by results of examinations of aqueous humor and serum of patients with aphakia where the ratio of ascorbic acid in the aqueous humor to that in the blood was extremely high. The authors conclude that the mechanisms involved in the production and maintenance of ascorbic acid in aqueous humor are not dependent on the presence or the condition of the lens. Linnér arrived at similar conclusions [49]: In continuation of his well-known studies on ascorbic acid as a test substance for measuring relative changes in the rate of plasma flow through the ciliary processes, he examined the ascorbic acid concentration in the aqueous humor and plasma of patients with normal lenses, senile cataract, and unilateral aphakia. Above the plasma saturation level, the concentration of ascorbic acid in the aqueous humor was of similar magnitude in cataractous and in normal eyes, nor was any difference discovered between the two eyes of patients with unilateral aphakia. In a group of such patients, the two eyes were compared below saturation level, and a significant difference in the ascorbic acid concentration in the aqueous humor was noted, indicating that the rate of plasma flow through the secretory part of the ciliary body is lower in eyes with senile cataract than in eyes with normal lenses.

Green, Capper, Bocher, and Leopold [50] used the method of Krebs and Roughton to measure the effect of acetazoleamide (Diamox) on the carbonic anhydrase activity of extracts of rabbit ciliary body and iris in order to study the possible influence of the enzyme on the secretory mechanism of these tissues. The possibility of introducing errors into the results by the carbonic anhydrase activity of the residual blood in the tissues was ruled out in preliminary tests. Fifteen minutes after the intravenous injection of 30 mg. of acetazoleamide per kilogram, carbonic anhydrase activity was inhibited as much as 75% and maximum inhibition was reached in 30 minutes. The rate of recovery of activity was slow; in 17 hours only 60% of the activity was restored. According to Lepri and Montanari,[51] parenteral injection of a single dose of 7 mg. of corticotropin in normal rabbits did not modify substantially the corticoid content of the aqueous humor. After the rabbits received injections of 7 mg. daily for a period of 10 days, a significant rise in the corticoid content was observed. In contrast to general experimental experience, Arimura [*52] observed a decrease in the permeability of the blood-aqueous barrier after injection of corticotropin in rabbits and an increased antibody formation in the aqueous humor. Lepri [53] demonstrated that the ascorbic acid level of the normal aqueous humor of the rabbit is not altered significantly by parenteral (5 mg/kg. for five days) or subconjunctival (7.5 mg/kg.) injections of cortisone. As mentioned above, the same author

had noted a lowering of the ascorbic acid content of rabbit aqueous humor after treatment with corticotropin. The differences may result from a direct effect of corticotropin on the titer or from mediation of corticoids which are different from cortisone. The effect of Dibenamine and some of its congeners on the turnover rate of various compounds of the rabbit aqueous humor was investigated by Harris,*[54] who reported on this study at the International Congress. The author did not notice an effect on secretion of ascorbic acid. The fall of the bicarbonate concentration paralleled that observed in arterial plasma. The steady-state ratio of monosaccharides was not altered by the pharmacological agent. The rate of transfer of glucose from plasma to aqueous humor appeared to be decreased. There seemed to be several explanations for this phenomenon. In this reviewer's opinion, the fall of general blood pressure and the depression of the heart observed in experimental animals after intravenous injection of Dibenamine may be related to the effect under discussion.

Siliato [55] injected rabbits in groups of four animals with adenosinetriphosphate (ATP), 1 mg/kg., intravenously, subcutaneously, and retrobulbarly, and studied the fluorescein permeability of the blood-aqueous barrier 1 and 15 minutes later. The dye appeared earlier when ATP was used. The retrobulbar injection was most effective. Control experiments were not reported. Giardini and Bagolini [56] describe the results of experiments on the aqueous humor of rabbits in which the fluorescein-appearance time was shortened by a few minutes and the concentration slightly increased after sodium pentobarbital (30 mg/kg.) was injected intravenously. As the series consisted of only eight animals, the author's conclusion of a pentobarbital-induced vasodilating effect at the barrier requires further study. Sapuppo [57] inferred from the results of experiments on 12 normal human subjects that light stimulation of one eye raises the permeability of the blood-aqueous barrier for fluorescein in the second eye, which was kept in the dark. With respect to fluorometry, it should be mentioned that the sound principle of Goldmann for measurements of fluorescence in the aqueous humor of the living eye was utilized by Langham and Wybar [58] in the construction of a fluorophotometric apparatus that fits into the Haag-Streit slit lamp and allows objective measurements of the degree of fluorescence in the anterior chamber. Hummelt [59] used radioactive penicillin which contained S^{35} to test the penetration of the antibiotic from the blood stream into the aqueous humor. The increase in concentration caused by paracentesis or experimentally induced severe inflammation is confirmatory evidence for old experimental results. Koch [60] elaborated on the differences between the macro- and microappearance (crystal and crack formation) of dried drops of primary and secondary aqueous humor of rabbits. Drying and lamellating cracks were seen in the drops of aqueous humor with high protein content. Extensive crystal formation characterized the microscopic picture of dried drops of primary aqueous.

LENS

Dische and Borenfreund [61] determined the nature of the constituents and the quantitative composition of polysaccharides in the bovine lens capsule. They used a series of spectrophotometric methods which had been elaborated by Dische, and which afford greater specificity and accuracy than previous methods. At variance with results reported by Pirie, the polysaccharide which is combined with capsular protein was demonstrated to consist of galactose and glucose in a ratio of 3:2. Chromatographic separation of these two hexoses by a method which does not change the ratio between the two constituents of the polysaccharide confirmed this result. It might be mentioned here that Glegg, Eidinger, and Leblond [62] analyzed hydrolysates of the whole lens capsule by paper chromatography and identified glucose, galactose, mannose, and fructose in the chromatogram; glucuronic acid was absent. Since no quantitative data are given, it is

difficult to evaluate the results referring to mannose and fructose, which were not detected by other investigators. Analysis of the various parts of the capsule by Dische and Borenfreund showed a difference in the absolute amount of polysaccharides and also in the ratio of polysaccharides to protein. The protein of the periphery was combined with much less of the polysaccharides than the protein of the central portion. The carbohydrate metabolism of the bovine lens capsule was studied by Dische and Ehrlich,[63] who incubated the preparations with glucose, glucose-6-phosphate, and fructose 1-6-diphosphate. The two esters were broken down at a similar speed aerobically and anaerobically along the usual glycolytic pathways. The breakdown also occurred when the epithelium had been scraped off, although at a lower rate. The fact that the metabolism of glucose by the capsule requires ATP indicated that phosphorylation of glucose to hexose-6-phosphate is the initial step in the breakdown in the capsule. At the incubation temperature of 37 C, ATP in the isolated lens capsule is rapidly dephosphorylated. At the International Congress, Dische *et al.*[64] reviewed some of his work on the metabolism of the lens capsule. He pointed out that 25% to 40% of the ribose of nucleotides disappeared in two hours under anaerobic conditions without the appearance of hexose-6-phosphate. This suggests that a different pathway for ribose metabolism is characteristic for the lens capsule.

Previous examinations of the lens capsule with polarized light demonstrated that cross sections of cattle lens capsule possess birefringence. Further study suggested that the capsule is a composite body, formed by lamellae bound together by a cement substance. In a report on their recent investigation, Bairati and Grignolo[65] described electron-microscopic images of cattle lens capsules. They were interpreted as indicating a lamellar structure consisting of submicroscopic layers embedded in a granular substance which exhibits slight metachromasia and is digested by hyaluronidase and oxidized by the periodic acid-Schiff reaction. Results of treatment with trypsin, hyaluronidase, and pepsin point to the composition of the single lamella as a birefringent protein and a material sensitive to hyaluronidase action.

Paper electrophoresis of water-soluble proteins of the lens was carried out on material obtained from the eyes of the human, monkey, horse, cattle, antelope, rabbit, and rat. The data reported in brief by François, Wieme, Rabaey, and Neetens[66] show that in young human eyes four protein fractions are discernible, in contrast to one in the eyes of older persons. Surprisingly, the composition of the nuclear and cortical proteins appeared to be the same in the human, while in the lenses of other species differences were noted. Gemolotto[67] analyzed electrophoretically the lens and aqueous humor of rabbits in which retinal degeneration had been produced by injecting sodium iodate 15 months previously. The resulting increase of glucose and decrease of ascorbic acid content had returned to normal at the time of analysis. The ratio between fast-moving and slow-moving fractions of the water-soluble lens proteins increased. The same author described[68] the electrophoretic pattern of lenses of rabbit eyes in which retinal degeneration was produced by sectioning of the optic nerve in five experiments. The changes were similar to those reported in the previous series, and consisted in an increase of the fast-moving protein fractions and a decrease of the slow-moving protein fractions. The description of the technical details was omitted, making it impossible to evaluate the results.

An investigation was undertaken by Nordmann, Mandel, and Achard[69] to determine in paired calf lenses the effect of certain inhibitors on anaerobic and aerobic metabolism of carbohydrates. Experimental and control lenses were maintained at 37 C in a perfusion medium consisting of dextrose-Tyrode solution with the addition of the inhibitors *d*-1-glyceraldehyde, monoiodoacetic acid, sodium fluoride, phloridizin, fluoroacetic acid, or sodium malonate. Osmotic

pressure and pH were controlled. Control lenses did not show any opacities in 24 hours, while the experimental lenses developed opacities in from 2 to 24 hours, depending upon the inhibitor used. The degree of lenticular opacification was established and correlated with the lactic acid and ATP values obtained by the examination of the lenses and surrounding fluids in both sets of experiments. The results bear out the fact that blocking of the breakdown of sugars is followed by cataract formation. Although the site of action is different, all metabolic poisons produced marked opacification of the lens. The cataract developed most rapidly when the block affected the anaerobic phase and less quickly when the citric cycle was involved. Christiansen and Leinfelder (1953),[70] who studied the problem of respiration in the lens, have expressed the opinion that this process is nonenzymatic in nature. This conclusion is based on the maintenance of normal oxygen consumption in spite of boiling the lens. Nordmann, Mandel, and Izraelewicz [71] showed in a recent study that oxygen consumption in the boiled lens is not of the same nature as that in the fresh lens. While oxygen consumption of the boiled lens was notably greater than that of the fresh lens, the consumption of glucose and the production of lactic acid were negligible under aerobic and anaerobic conditions in the boiled lens, as compared with the processes of the fresh lens. The addition of lead acetate to the preparation lowered the oxygen consumption of the boiled lens to less than that of the fresh lens. It is concluded, therefore, that the consumption of oxygen by the boiled lens is an auto-oxidative process and dependent on the denaturation of proteins. It cannot be compared with respiration in the fresh lens. Intermediary reactions of the metabolic pathway in anaerobic utilization of carbohydrates by the rabbit lens were the subject of an excellent contribution by Green, Bocher, and Leopold.[72] Only the most important results can be mentioned here. Extracts of lens homogenates obtained from young rabbits served for metabolic studies on the anaerobic formation of lactic acid when ATP, inorganic phosphate, hexokinase, and various hexosephosphates were added to the suspension medium. Glucose-1-phosphate and glucose-6-phosphate appeared as the first products of glucose metabolism. A relative deficit in hexokinase was considered a limiting factor in the glycolysis of lens extracts. In a second study,[73] the investigators examined the anaerobic metabolism of fructose diphosphate in the lens extract. The slight ability of this ester to give rise to lactic acid was potentiated by the presence of suitable phosphate acceptors, an observation which suggests identity of the pathway of fructose diphosphate breakdown with that in other tissues and a role of this ester as intermediate in glucose metabolism. Mandel and Klethi [74] demonstrated the presence of succinic acid dehydrogenase in calf lenses by the usual Thunberg procedure and showed also that the reduction of succinic acid is inhibited by 0.01 M malonate. In addition, they observed the reduction of potassium ferricyanide in the presence of lens extract and a preparation of succinic acid dehydrogenase from sheep heart, which may indicate the presence of succinic acid in quantities of about 1.68 mg/100 gm. Mandel and Izraelewicz [75] identified hexokinase and phosphofructokinase in extracts of calf lenses and attempted to demonstrate the presence of aldolase and triose phosphate dehydrogenase. The paucity of experimental results, however, makes evaluation of the data concerning the last two enzymes difficult. Results of previous studies by Pirie, van Heyningen, and Boag [76] have shown that the earliest change detected in the rabbit lens during development of x-ray-induced cataract is a diminution of glutathione. The activity of the enzyme glutathione reductase is correspondingly diminished, but the activity of four other enzymes, not thought to depend on functional sulfhydryl groups, is unaffected. Since protein sulfhydryl is also diminished during a later stage, further studies by the same investigators [77] have been made on

three enzymes and one coenzyme which depend on SH groups for their activity. These enzymes are glyceraldehyde-phosphate dehydrogenase, glyoxalase, an acetaldehyde oxidase, and coenzyme A. The results indicate that enzymes which are dependent on SH groups are affected by radiation before those not so dependent. Since change in enzymatic activity occurs later than change in glutathione concentration, the concentration of enzyme SH groups may possibly be obtained at the expense of glutathione. It is also suggested that, if glutathione acts as a stabilizer of protein SH, the enzymes may become reversibly inactivated when glutathione of the lens falls below a certain level. The loss of weight noted in the irradiated lenses may be due to a failure in protein synthesis.

Radiophosphorus was used by several investigators, who studied the exchange of the indicator across both lens surfaces and its passage through the lens. Palm [78] incubated fresh rabbit lenses with one surface of the lens bathed in the animal's own fresh aqueous humor to which P^{32} had been added. After 4 to 10 minutes of incubation, the lenses were analyzed as a whole and also after division into anterior and posterior halves. The results indicate that exchange can take place readily across both surfaces. Analysis of the lens halves showed good exchange through the tissues. The participation of the phosphate, which has penetrated from the outside, in the lens metabolism is independent of the site of entrance. Organic phosphate, which is hydrolyzed with more difficulty than ATP, was increased directly beneath the anterior surface. The differences in concentration in various parts of the lens could be explained as differences in the phophorylation of carbohydrate, which would lead to variation in the content of hexose and triose phosphates. Havener and Knorpp [79] injected rabbits intravenously with P^{32} in the form of acid phosphate. At set time intervals, the lenses were removed, divided into anterior and posterior halves, and analyzed for P^{32} uptake. In some instances, the epithelium was scraped off and its radioactivity measured. The activity exceeded by a factor of 100 that of the rest of the lens. Under the conditions of the experiment, however, no significant difference in the concentration of P^{32} in the anterior and the posterior portion of the lens was demonstrable. This result is surprising to the reviewer, since radioautographs of lenses from rabbits injected with P^{32} show, at various time intervals, a photographic effect in a larger area in front of the equatorial plane than behind it.

The hydration of the lens and cornea has been thought by various investigators to depend on metabolic activity as the energy source for active transfer mechanisms. Schwartz, Danes, and Leinfelder [80] dealt with this problem in an interesting study on cattle lenses and rabbit corneae. They incubated these tissues in the synthetic medium of Merriam and Kinsey and investigated the effect of temperature variations, anoxia, depletion of glucose from the medium, pH changes, and enzyme poisons on fluid uptake by the tissues. Thus, the approach was similar to that followed by Davson in investigating corneal hydration. They concluded that tissue metabolism plays an active part in maintaining a steady state of water content, as the experimental results showed that metabolic energy is required to prevent tissue dehydration and also, in the lens, to restore to the original level the weight, which had previously increased during anoxia. Moreover, the authors observed that anoxia and hypertonic stimuli produce a shift in metabolism toward increased glycolytic activity, suggesting the existence of a Pasteur effect in lens and cornea. In a continuation of previous work, Harris, Hauschildt, and Nordquist [81] studied the role played by glucose in the reversible cation shift in the lens. The low-temperature-induced shift was reversed maximally at a glucose concentration of 200 to 350 mg. per 100 cc. At higher concentrations recovery declined markedly, and adjustment of the osmotic pressure of the 200 mg. per 100 cc. glucose medium to equal that of a 500 mg. per 100 ml. solution did not reduce the degree of cation recovery. ATP overcame

completely the deleterious effect of high glucose concentrations. It may be, then, that glucose depletes lenticular ATP to a harmful degree. Insulin has no effect on the cation transport, and l-glutamic acid and sucrose were found to enhance the cation reversal in the presence of adequate glucose. However, by themselves, they could not replace glucose as a source of energy. The possible implications of these results for cataractogenesis in diabetes are obvious. The results of this important investigation prompted the authors to study the nature of the movement of glucose into the lens.[82] Rabbit lenses were incubated in media having a high glucose content, and the depletion of glucose from the media was measured after various enzyme inhibitors (fluoride, iodoacetate, dinitrophenol) had been added. Although in no instance was a higher concentration reached in the lens than was present in the surrounding fluid, the data obtained pointed to a metabolic mediation in the movement of glucose from the surrounding fluid into the lens. The site of the barrier was not conclusively established, but it seems to be located either at, or in close proximity to, the lens capsule. The extensive work of Harris and co-workers was reviewed in part by Harris *[83] on the occasion of the International Congress, where he discussed factors which influence the electrolyte and water balance of the lens.

In a study of galactose cataract in rats, Hörmann [84] arrived at the conclusion that inhibition of enzymatic carbohydrate breakdown has to be considered as the cause of the lenticular opacification. When animals on a diet containing 30% galactose were treated at intervals of four days with subcutaneous injections of cocarboxylase in a dose of 5 mg., the lens opacities were inconspicuous and regressed with increase of the enzyme to 10 mg. In all control animals cataracts developed. Patterson [85] investigated the effects of partial starvation on the development of cataract in rats with alloxan diabetes. A control group was permitted to eat and drink ad libitum. After three weeks, the experimental group was starved for a period of 40 hours each week and blood sugar levels were determined twice—once on the day of starvation and once on a feeding day. Withholding food effected a fall in the blood glucose of about 200 mg/100 cc. Cataract formation did not take place in those rats with a nonfasting blood sugar of 450 mg/100 cc. or less but did develop in animals with severer diabetes, i. e., with nonfasting blood sugar levels of 450 to 550 mg/100 cc. Patterson's *[85] hypothesis of a dependence of carbohydrate cataract formation in rats on high blood levels of galactose, xylose, glucose, and arabinose has not been confirmed uniformly. Alloxan-induced cataract in rabbits was encountered by Babel [87] after prolonged glycemia had developed. Formation of subcapsular vacuoles was the predominant change. They disappeared when the metabolic disturbance did not exceed four to six weeks and appeared again upon repeated injection of alloxan. While investigating the effects of cholinesterase inhibitors on the central nervous system, Diamant [88] observed that the guinea pigs used in the experiment occasionally developed cataract. The inhibitors were organophosphorus compounds used in the treatment of conditions requiring parasympathicomimetics, e. g., glaucoma. The animals received intracarotid injections of isofluorophate (diisopropyl fluorophosphate; DFP), tetraethylpyrophosphate (TEPP), and *p*-nitrophenyldiethylphosphate (Mintacol). Cataract occurred in 31 cases, in 20 of which isofluorophate was the causal agent. The cataracts were localized chiefly in the central parts of the anterior surface of the lens and, in many cases, were reversible. No detailed descriptions of the cataract changes were given, and the nature of the surprising results remains unexplained. Martinet, Baron, and Guillon [89] compared the sodium and potassium contents in normal and in cataractous lenses and in lenses of fetal and maternal rats. In addition, fetal cataract was produced by thyroxin injection during gestation. In cataractous lenses, the sodium content was increased, while, according to these investigators, the potassium concentration remained unchanged, a result

which is difficult to understand. Soya [*90] found that naphthalene impedes the synthesis of vitamin C from di-ketogulonic acid in the crystalline lens. The manganese ion accelerated synthesis, while the calcium ion impeded it. According to Ishihara,[*91] vitamin C prevents the development of naphthalene cataract. β-Naphthoquinone is excreted in the urine of naphthalene-treated rabbits. Cataract formation by β-naphthoquinone can be prevented by 5-oxyanthranilic acid. Alajmo and Ambrosio [92] set lesions at the limbus of rabbits by diathermic coagulation and measured weight changes of the lens 1, 7, and 24 hours and 1 week after treatment. Increase in the weight occurred at the 24-hour interval. The authors explained the weight gains as caused by permeability changes in the lens capsule.

Kleifeld, Fuchs, Hockwin, and Arens [93] continued the studies of the effect of electric stimulation on the transparency and thickness of lens fibers, which Kleifeld had observed originally by phase microscopy.[94] They determined the lactic acid, vitamin C, glutathione, glucose, and phosphate contents of the lenses of calves, swine, and rabbits. The material was kept under liquid petrolatum and exposed to 10 electric stimuli per second for 30 minutes (10 ma.; duration 40 msec.). The temperature rose about 4.5 degrees (C) during stimulation. The concentration of the tested substances, with the exception of glucose, decreased. Similar results were obtained in experiments on the intact rabbit and swine eyes. The decline in the lactic acid content is explained as diminished formation of the compound, not by changes in the conditions for diffusion.

Agarwal and Mehra [95] tested patients who had undergone cataract operations for skin sensitivities to lens antigen and to cysteine, and they claim to have observed a close parallelism with respect to both antigens. The responses to the latter are not tabulated separately, and the incidence of iridocyclitis after extracapsular extraction and the degree of sensitization showed no proportionality. For these reasons, the evidence presented appears insufficient to support the conclusion that cysteine injection is a simple way by which to determine sensitivities to lens protein and to diagnose postoperative inflammatory changes as phacoanaphylactic.

VITREOUS HUMOR

Varga and Balazs [96] studied liquefied and concentrated samples of cattle vitreous electrophoretically and with the analytic ultracentrifuge. With the former method, two to four boundaries were separated, depending upon the pH of the buffer. The sedimentation boundaries which showed up in the ultracentrifugal pattern were identified by treatment with enzymes. The slowly migrating component disappeared after the addition of hyaluronidase to the sample and was intensified after the addition of hyaluronic acid. Liquefied vitreous, which had been filtered through the Seitz K-7 asbestos filter, showed only one boundary, similar in shape and sedimentation constant to the slow-moving sedimentation boundary of the unfiltered vitreous. It was thought to be hyaluronic acid or a hyaluronic-acid protein complex. The fast sedimentation peak disappeared after trypsin and papain treatment. For this reason, and because of its electrophoretic behavior, this component was identified as albumin. The third and fourth boundaries of the electrophoretic pattern were considered as belonging to γ-globulins. It should be noted that the ultracentrifugal pattern did not reveal the presence of globulins. Since these proteins have been demonstrated in the vitreous by various investigators with chemical methods, this experimental result of Varga and Balazs requires further clarification. In ultraviolet spectrophotometric studies on cattle vitreous, Balazs [97] noted an absorption maximum at 265μ in the pH range from 5 to 10, which he considered to be caused by ascorbic acid. An absorption maximum at 277μ in the dialyzed vitreous is caused by protein. On the basis of the tyrosine and tryptophan values, the globulin type was judged to be prevalent. An A/G ratio of 0.46 was estimated. Among the techniques used and elaborated by Brunish, Rowen, and Irvine,[98] in a fundamental study on the pro-

teins and hyaluronic acid of beef vitreous humor, the quick-freezing procedure deserves special mention, since it has the advantage of better preserving the physical and chemical integrity of the constituents of the gel. Electrophoretic studies on minced, filtered, and concentrated vitreous disclosed the presence of hyaluronic acid and at least three protein fractions. The mobilities of the major protein components corresponded to those of bovine serum albumin and α- and β-globulin. These results are at variance with those obtained by Varga and Balazs [96] with a similar technique but confirmed the low albumin-globulin ratio which Balazs estimated by spectrophotometric methods.[97] This ratio suggested to the investigators that these proteins did not derive from serum by a simple diffusion mechanism. Of great interest are the experiments on enzymatic degradation of vitreous filtrate and sodium hyaluronate by hyaluronidase, as they deal with the controversial subject of the chemical composition of the hyaluronic acid molecule. The authors found an enzyme-resistant, nondialyzable fraction which has the same elemental composition as the total molecule, whereas Kaye and Stacey postulated a core consisting primarily of glucosamine residues and other workers reported theoretically maximal hydrolysis. The above-mentioned filtrates and the solution of purified sodium hyaluronate were studied by means of streaming birefringence and light-scattering measurements. It was concluded that hyaluronic acid has a molecular rate of 400,000 to 1,000,000 and exists as an elongated, thread-like molecule containing many hydroxyl groups and a charged carboxyl group. The explanation offered for the turgor of the vitreous body is the binding of extremely large quantities of water by the easily accessible hydrophilic groups of the molecule. In a review paper, Balazs [*99] discussed the major macromolecular components of the vitreous (fibrous proteins, hyaluronic acid, glycoprotein, and blood proteins) and the role which they play in the structure of the gel. Age-induced changes in the structural characteristics of the gel, dependence of the physical state on the water-binding capacity, and factors which influence the movement of ions and charged molecules through the vitreous were enumerated. On the occasion of the International Congress, Varga [*100] explained some theoretical points pertaining to colloid chemical cha acteristics of the vitreous as a multicomponent fine gel.

Since little is known of exchange conditions at the posterior lens surface, Cristiansson and Palm [101] undertook a study in which they determined the rate of removal of phosphate ions in the most anterior layers of the vitreous body. The inorganic phosphate contents of blood serum, aqueous humor, and different parts of the vitreous body were measured. The vitreous was frozen and separated into a posterior part, a layer adjacent to the posterior lens surface, and a peripheral anterior (ciliary) part. The inorganic phosphate content of the vitreous (5.7 γ/gm.) is considerably lower than that of the aqueous (23.1 γ/gm.), while within the vitreous body the content is higher in the ciliary portion than in the remaining parts. Dynamic experiments were performed with the injection of P^{32}-labeled Na_2HPO_4. The specific activities of various parts of the vitreous were illustrated by radioautogram. The experiments indicate that the ciliary part is renewed at a higher rate than the remainder, but in general the renewal in the vitreous body proceeds slowly and uniformly. In a brief report, Wootten, Young, and Williams [102] describe a quantitative analysis of the amino acid composition of the vitreous humor. By means of the ion-exchange chromatographic technique, it was possible to identify 17 amino acids; several as yet unidentified ninhydrin-reactive substances were also revealed. Young and Williams [103] devised a gentle method for preparing a gelatinous protein of the vitreous body. Extraneous protein was removed by papain. Ash-free material obtained by repeated washing of the vitreous showed an amino acid pattern similar to that of gelatin, that is, high proportions of aminoacetic acid, proline, and hydroxyproline. No methionine was detected.

Planten and Hoppenbrouwers [104] failed to produce changes in the intraocular pressure of rabbits by injecting 0.1 ml. of saline containing 5 V.R.U. of hyaluronidase into the vitreous (10 experiments). The contralateral control eyes received an injection of 0.1 ml. of saline. In the concentration used, the enzyme did not cause liquefaction, nor, apparently, did it influence the absorption of blood from the vitreous. The small number of experiments and the lack of histological observations create some uncertainty as to the validity of the conclusions.

RETINA

Reis discussed the disadvantages of previous histochemical methods of localizing alkaline phosphatase in the retina and artifacts caused by these methods. In the present study,[105] he applied to the bovine retina the method devised by Gomori (1951) and modified by Gregg and Pearse (1952). This method depends upon the coupling of the α-naphthol produced by phosphatase hydrolysis of α-naphthyl phosphate with the diazotate of 5-chloro-*o*-toluidine. A red dye is produced which is easily distinguishable from melanin pigment and does not diffuse readily, thereby preventing the formation of artifacts. According to the results of this procedure, the phosphatase activity is localized in the vascular endothelium of the retina, in the whole of the uvea, and in the choroid plexus. The author suggests that the site of its localization corresponds to a hypothetical "blood-aqueous" barrier. According to Weitzel, Strecker, Roester, Buddecke, and Fretzdorff,[106] the tapetum cellulosum of seven examined species of Carnivora has a high content of zinc (up to 11.8% in the tissue of fox), in contrast to the relatively low values which were found in Herbivora. Injection of dithizone, which forms zinc complexes, caused blindness and retinal detachment in dogs within 24 hours. Dithizone injection also reduced the zinc content of the choroid and the tapetum to almost zero levels. The authors consider the retinal detachment to have been caused by primary damage to the choroid. Glycogen determined by the PAS method was found by Rabinovitch, Mota, and Yoneda [107] in the paraboloid of accessory cones and in the characteristically shaped paraboloid of the rods. No difference in glycogen distribution could be discerned in the visual cells of dark-adapted eyes. Pentose-polynucleotides were revealed when the gallocyanin-chromalum technique was applied. Basophilia, removable by ribonuclease or perchloric acid digestion, were present in the inner segments of both the chief and the accessory cones except in the ellipsoid region. Hirano,*[108] using histochemical techniques, studied arginine distribution in the retina. In the neuroepithelium, the cones contained a greater amount of arginine than the rods, and the inner segments of the visual cells had a greater amount than the outer segments. The ganglion cells and the nuclear layers also gave a positive arginine reaction.

Heare and Kerly *[109] demonstrated in extracts of cattle retina three glycolytic enzymes: hexokinase, phosphohexoseisomerase, and 6-phosphofructokinase. *D*-glucose, mannose, fructose, and glucosamine were phosphorylated by these retinal extracts. The inhibition of fructose phosphorylation by glucose and glucosamine suggests that these three sugars are substrates of a common hexokinase, such as occurs in yeast, in contrast to the separate gluco- and fructokinases, which occur in muscle. Röe [110] compared oxygen consumption of the retina with that of the cerebral cortex, the metabolism of which was thought to be very similar to that of the retina. The author has shown that results of studies on metabolism of the retina should not be regarded as applicable to metabolism of the brain cortex, since the optimum pH for brain respiration lies between 9.0 and 9.5, while that of the retina is between 7.4 and 8.0. In a report on their latest study, Becker, Allen, Winter Maengwyn-Davies, and Friedenwald [111] discussed recent experimental and clinical observations which point to a relation between diabetic retinopathy, an increased adrenocortical activity, and vitamin B deficiency. They then presented the results of a series of experiments which they conducted on cortisone-

treated rabbits, which were divided into three groups: One group was maintained on a diet of Sherwood rabbit pellets; in the second group, vitamin B_{12} (5 mg/ton) and chlortetracycline (Aureomycin) were added; in the third group, 100γ of vitamin B_{12} was given daily with the unsupplemented diet. The experiments were terminated on the 21st day. Kidney lesions occurred in all animals when vitamin B_{12} was withdrawn. Supplements of the vitamin reduced the incidence to 32% regardless of dose. In 22 patients with diabetic retinopathy vitamin B_{12} was excreted in an average amount of 19 mg., as compared with 9.6 mg. in nondiabetics and 4.2 mg. in patients with diabetes unaccompanied by retinal involvement. Earlier work of Becker, Lang, and Chow [112] had shown that, following an intramuscular test dose of vitamin B_{12}, diabetic subjects with retinopathy excreted significantly more of the vitamin than nondiabetics, while diabetics without retinopathy excreted considerably less than nondiabetics. In their present work,[112] Chow, Rosen, and Lang used a method which permits estimation of as little as 10γ of vitamin B_{12} activity and compared the serum levels in diabetics with and without retinal lesions. They observed that those patients with retinal lesions have a much higher activity ($292 \pm 24\gamma$) than those without retinopathy ($162 \pm 18\gamma$). The investigators conclude that vitamin B_{12} is somehow fundamentally involved in the pathogenesis of retinopathy.

Becher [114] has interpreted morphological changes in the large multipolar visual cells and smaller ganglion cells of the retina of man and animals as indicative of secretory activity and distinguished various stages of this "neurosecretion." Liquefaction of the granules in the periphery of the cytoplasm led to vacuole formation and was followed by discharge of these vacuoles. The nucleus and nucleolus were also involved in the secretory changes. Theoretical considerations on the significance of this process are advanced. PAS-positive granules were found by Serpell [115] to occur normally in association with proliferating mesenchymal cells in the developing retinal vasculature in man, rabbit, and cat; they occur pathologically in the retina in the early stages of retrolental fibroplasia in man and the kitten. Histochemical observations led to the conclusion that these polysaccharide granules consist of glycogen. Pigment granules obtained by mechanical destruction and subsequent centrifuging of pigment epithelium of human retinae were examined by Binder and Orth.[116] Under 35,000 × magnification, spindle-shaped particles 2μ to 3μ in diameter and spherical granules 1μ in diameter were distinguished. Zonular fibers examined in a similar way varied in thickness from 0.5μ to 12μ and showed length striation.

Rabinovitch, Mota, and Yoneda [117] observed in the retina of young chicks degenerative changes setting in quickly after intravenous injection of iodoacetate in a dose of 40 mg/kg. After eight hours the nuclear layers appeared to be reduced in thickness, but pyknosis of the ganglion cells was noted after 30 minutes. Hasegawa *[118] reported that chronic carbon monoxide intoxication produced retinal degeneration in hamsters when the experimental animals were exposed to CO gas (0.03%-0.05%) eight hours daily for 20 to 110 days. Pavia [119] produced hypercholesterolemia in five dogs by maintaining the animals on a cholesterol-rich diet for four months and noted as fundus lesions crystalline deposits in the retina and arteriolar constriction. Kubota and Morikawa *[120] stated that corticotropin injection in the rabbit (4 mg. daily for three days) was followed by a decrease in carbohydrate breakdown in the retina of about 15.3%. Aerobic glycolysis decreased about 12.7%.

VARIOUS OCULAR AND ORBITAL FLUIDS AND TISSUES

Several studies on tear fluid deal with the lysozyme content of human tears. The separation of proteins in this fluid by means of paper electrophoresis was successfully carried out by Caselli and Schumacher,[121] who observed four components: three migrating in the electric field to the anode and one to the cathode. The last-mentioned,

identified as lysozyme by its lytic action on Micrococcus lysodeicticus, represented one-third of the tear proteins. The other fractions corresponded roughly in their mobilities to serum proteins. Miglior and Pirodda [122] and McEwen and Kimura,[123] using a similar technique, obtained only three boundaries, possibly because of a shorter experimental time. In other respects, they confirmed the results of Caselli and Schumacher. Smolens, Leopold, and Parker [124] conducted similar experiments and found four components. It can be said, then, that lysozyme can be separated from tears as a uniform basic protein. Evaluation of differences in lysozyme concentration under pathologic conditions and the contradictory results reported are not unexpected in view of the unknown contamination of tear fluid by secretion from the conjunctiva, and overproduction and dilution of tears under the influence of lacrimator stimuli.

The cholinesterase activity of the lacrimal and salivary glands varied, according to Hama,[125] upon electric stimulation of subthalamic nuclei. It increased when the stimulus was applied to a ventral medial zone and decreased when the lateral area was stimulated. Takagi *[126] described the periodic acid-Schiff reaction in the lacrimal gland and the dependence of this reaction on the hexosamine content of the tissue.

Smelser and Ozanics demonstrated,[127] in investigations on the nature of the exophthalmos-producing principle in pituitary extracts, that the degree of exophthalmos in thyroidectomized guinea pigs is proportional to the amount of the crude extract injected. Relatively pure extracts of gonadotropin, follicle-stimulating hormone, corticotropin, prolactin, or growth hormone did not produce proptosis. The effects of hypophyseal extracts of cattle, pig, sheep, and horse were analyzed and tested. The exophthalmos-producing potencies in no way reflected the thyrotropic hormone content. The study covered several other phases of the intriguing problem, such as the influence of various hormonal extracts on orbital fat, eye muscles, Harderian gland, and ovarian and adrenal glands, to which only brief reference can be made in this review. In an additional study,[128] the authors investigated the effects of combinations of pituitary factors on the orbital tissues. In confirmation of earlier conclusions, at least two anterior pituitary factors were found to be involved in the production of experimental exophthalmos. Corticotropin and adrenal steroids (cortisone) increased the exophthalmos produced by anterior pituitary extracts, whereas a combination of cortisone with large quantities of either growth hormone, prolactin, or gonadotropin, was ineffective. It was shown that exophthalmos, edema, and hypertrophy of the orbital fat are produced by concomitant administration of cortisone and small quantities of thyrotropin. This experimental exophthalmos can hardly be explained on the basis of the quantities of thyroid-stimulating hormone administered, since combined injections of several purified anterior pituitary hormones containing only traces of thyroid-stimulating hormone are more effective than equal quantities of a purified thyrotropin. It can be said, then, that experimental exophthalmos appears to be the result of the synergistic action of two or more substances.

Praglin, Spurney, and Potts [129] used electroretinography as a tool in a series of studies conducted in the research laboratories of Western Reserve University on the toxicity of methanol in experimental animals, and to distinguish the effects of methanol, formate, and formaldehyde. The dosage necessary to abolish the b-waves in the electroretinogram was of similar magnitude for formate and methanol (0.025 to 0.300 M/kg. and was much smaller for formaldehyde (0.007 M/kg.). Since a dose of 0.005 M/kg. of methanol can cause blindness in the human, it is concluded that the poisoning is brought about by the conversion of methanol to formaldehyde. Gilger and Potts [130] conducted experiments on rats, rabbits, dogs, and rhesus monkeys to determine whether methanol-induced acidosis causes visual damage. Acidosis developed regularly in rhesus monkeys but rarely in non-

primates. Toxicity, symptomatology, and ocular pathology of methanol poisoning in the monkey resembled that in man. Potts demonstrated in a subsequent study that [121] adequate doses of sodium bicarbonate may protect monkeys against twice the lethal dose of methanol. Some animals died, however, despite prevention of acidosis by sodium bicarbonate. In addition, treatment did not prevent the appearance of retinal edema.

Illustration of the profound effects of some styrylquinoline and cyanin dyes on certain metabolic processes (Peters, 1952) led to the supposition that these dyes would be useful in investigating metabolic processes related to aqueous humor secretions. Ballantine and Peters [122] injected styrylquinoline dye No. 350 into the common carotid of albino rabbits after ligation of the external branch. This procedure resulted in concentration of the dye in the ciliary epithelium, without destructive effect or inflammatory lesions. Consistent depression of intraocular pressure was produced in the treated eyes, along with decreased ascorbic acid and carbon dioxide concentrations in the aqueous. Oxygen uptake was depressed in about one-half the treated eyes, but this depression of oxidative metabolism does not explain the hypotension. Pressure remained lowered for two or three weeks, whereas the oxygen uptake returned to normal after four days. The ascorbic acid concentration was affected longer than the intraocular pressure. The fact that this dye inhibits oxidative phosphorylation at concentrations lower than those inhibiting oxidation suggests that inhibition of phosphorylation is responsible for the ascorbic acid and carbon dioxide effects, while the oxygen effect appears only in those eyes which accumulate higher amounts of the dye. A study was undertaken by de Roetth [123] to determine the factors influencing the glycolytic activity of the ciliary processes. He used the Warburg manometric technique under aerobic and anaerobic conditions to determine the glycolytic activity of the ciliary processes of 600 beef eyes. Optimal glycolytic activity was observed in a Krebs phosphate medium at a pH of 7.4 to 7.6. Iodoacetate and sodium fluoride considerably depressed both anaerobic and aerobic glycolysis. Glyceraldehyde inhibited anaerobic lactic acid production of this tissue, suggesting that glycolysis of the cilliary processes is mainly glucose glycolysis, and not glycogen glycolysis. The author demonstrated previously,[124] with regard to oxygen uptake, that the ciliary processes did show "stimulation metabolism" typical of secretory glands following the addition of parasympathicomimetic drugs. However, the effects of acetylcholine and physostigmine on aerobic glycolysis were negligible. The results of the investigation also indicate that anaerobic glycolytic activity of the ciliary epithelium approximates that of the retina.

Dunér, von Euler, and Pernow [125] determined the catechol amine content of various parts of the eye in an attempt to learn more about the extent and distribution of the sympathetic nerve supply to these structures. With elaborate assay methods, arterenol was demonstrated in appreciable amounts in the ciliary body, iris, and choroid. The concentration corresponded roughly to that found in other smooth muscle organs. Epinephrine was present in concentrations of 2.6% to 7.2% of the total catechol amine. The retina did not contain measurable amounts of epinephrine or arterenol. This might provide evidence against an adrenergic nerve supply to this tissue. Lens and vitreous were free of the catechol amine, and arterenol amounted to less than 0.2 γ/100 cc. in the aqueous humor. It is considered that this might have leaked from the adrenergic nerve endings of the anterior part of the uvea into the aqueous humor. In an earlier investigation, Pillat and Powers [126] had attempted to measure epinephrine and arterenol activity in the aqueous humor of cattle and cat eyes and were unable to demonstrate catechol amine in 10-times-concentrated samples. Arterenol was present in quantities of 0.1γ to 0.2γ/gm. of tissue in the ciliary

body and iris. With the bioassay technique, no epinephrine effect of the tissue extracts could be observed.

Quantitative analyses of various lipid fractions were made on different parts of the cattle eye by d'Asaro, Young, and Williams.[137] Ethanol-ether extracts of the samples were analyzed for total fatty acids, cholesterol, phospholipids, and choline-containing lipids. It was established that most of the fatty acid exists as essential lipid (total lipid minus neutral fat) and that cholesterol and phospholipids (80%) comprise most of the lipids determined in these tissues. The great differences noted in the lipid content of the cornea, lens, retina, iris, and ciliary body suggest a wide range of metabolic activity in these tissues. Oksala[138] studied spectrographically, after dry and wet decomposition of the organic matter, the trace metals in cattle cornea, sclera, lens, vitreous body, uvea, and retina. Barium was the most abundant of the metals, and its distribution did not differ significantly in the various tissues. Iron was found in relative high concentration in the uvea. In contrast to the results of earlier investigations, no differences were detected in copper distribution. Aluminum values were similar in extent to those of iron, with the lens slightly richer in this element than other parts. Zinc, silicon, molybdenum, silver, lead, and nickel were equally distributed in all examined tissues of the eye. Tin, also present in cornea and vitreous, was abundant in lens samples.

In a report on degenerative change in the rat visual pathway when thiamine and riboflavin deficiencies are combined, Rodger[139] described experiments on rats in which (1) thiamin was administered at inadequate levels, (2) riboflavin was omitted, and (3) the diet was moderately deficient in both vitamins. The survival time was reduced greatly in the thiamin-deficient group and moderately in the riboflavin-deficient rats. Signs of degeneration in the visual pathways were noted only in the thiamin-deficient animals. Furthermore, the results indicate that prolonged thiamin deficiency in conjunction with a predominantly carbohydrate diet causes optic atrophy. In a histochemical study of the skin of the lids, Morone and Sacchi observed[140] that sebaceous glands are more numerous and richer in lipids in the inner third of the lid. They are nonfluorescent and Hotchkiss-negative.

Dr. Zacharias Dische, Biochemist of the Department of Ophthalmology, Columbia University College of Physicians and Surgeons, evaluated many of the papers reviewed.

VISUAL PIGMENTS

Section Written by Ruth Hubbard, Ph.D., Biological Laboratories, Harvard University, Cambridge, Mass.

Rhodopsin: Synthesis, Physical Properties, and Concentration in Rods.—Previously in this journal I reviewed the mechanism of rhodopsin synthesis from a cis isomer of retinene, or vitamin A (neo-b), and the protein opsin.‡ Briefly, opsin in the dark condenses spontaneously with neoretinene b to form rhodopsin, which in the light bleaches to a mixture of all-trans retinene and opsin. Either retinene isomer is in equilibrium with the corresponding isomer of vitamin A. All these reactions have been carried out in a soluble purified system. Rhodopsin synthesis therefore requires a continuous supply of the neo-b isomer. Collins and co-workers have been exploring the synthesis of rhodopsin in retinal homogenates from frog and rat§ and now describe experiments performed with homogenized cattle retinas.[143] They find that a cattle retinal homogenate in phosphate buffer and supplemented only with all-trans vitamin A, or retinene, can synthesize rhodopsin. Of a number of co-factors tried, only pyridoxal phosphate enhances the reaction. The role of this co-factor is unclear. Irradiation also helps the regeneration, and the authors suggest that this may be due to an increase in the isomerization of all-trans retinene to neoretinene b.[144] The results of these experiments are, however, extremely variable, giving regenerations anywhere from 48% to 200% of the

‡ References 141 and 142.

§ Cf. Reference 142.

rhodopsin content of the unbleached control retinas. This points up the difficulty of getting clear-cut answers from as complex a system as a homogenate of whole retinas.

The sedimentation of cattle rhodopsin has been studied in the ultracentrifuge.[145] Hecht and co-workers had shown that frog rhodopsin in digitonin precipitates as a particle with a molecular weight of about 280,000. This is confirmed in the present study, but it turns out that this particle (or micelle) contains only about 40,000 gm-M (gram moles) of rhodopsin; the remaining 240,000 gm-M are contributed by digitonin (about 120 molecules). By determining the extinction and the concentration of micelles in a number of rhodopsin solutions, and knowing the molar extinction of rhodopsin per retinene unit ‖ and the molecular weight of the micelle (280,000), one can calculate that each micelle contains one molecule of retinene. The micelle, therefore, contains a single molecule of rhodopsin with a molecular weight of about 40,000, and carrying a chromophore composed of one retinene molecule. Hubbard calculates that a frog rod outer limb contains about 2.1×10^8 molecules of rhodopsin, which would give it an extinction at 500 mμ of 0.5 viewed end-on. Cattle outer limbs, being much smaller, contain about 4.2×10^6 molecules and have an extinction of about 0.037. Rhodopsin accounts for about one-fifth the protein of cattle outer limbs and an even higher proportion in the frog. It must, therefore, be an integral part of the structure of the outer limb.

Arden [146] has measured the extinction of dark-adapted frog rod outer limbs suspended in sucrose. From an estimate of the total volume of outer limbs in the suspension, he derives an extinction at 500mμ of 0.31-0.35 per rod end on. Denton ¶ measured the extinction of frog rods in situ by a photographic method and found a value of 0.7-0.75. This higher extinction has been attributed to an increased photosensitivity of rhodopsin in the rod in situ. This has now been confirmed experimentally by Hagins,[147] who finds that rhodopsin is about 1.6 times as photosensitive in the rod as in solution. This difference must be due in part at least to the orientation of rhodopsin in the outer limb. Schmidt,[148] many years ago, described the dichroism of dark-adapted rods (i. e., rods which contain rhodopsin), demonstrating that they absorb more strongly light polarized in a plane perpendicular to the rod axis. In end-on view, the rods absorb equally well in all planes of polarization. These observations have been confirmed and extended by Denton.# He finds that, on bleaching, the dichroism at 500 mμ (rhodopsin) disappears and grows stronger at 360 mμ (retinene), until it disappears there, too. He concludes that, after bleaching, retinene initially still maintains its orientation, but then becomes disoriented. Denton [151] has adapted this method so that he can measure the absorption spectrum of rhodopsin by plotting dichroism against wave length. The "dichroism spectrum" agrees perfectly with the well-known absorption spectrum of rhodopsin (λ_{max} at 500 mμ).

Metabolic Reactions of the Rod Outer Limb.—Arden * has observed the rhodopsin cycle in the isolated frog rod outer limb and finds that it follows the reaction scheme derived from analysis in homogenates and soluble systems, i. e., that rhodopsin bleaches in the light to retinene, which, in turn, is converted to vitamin A. Aged rods lose the capacity to convert retinene to vitamin A, due to a loss of enzymatic activity. His technique of measuring these reactions is interesting: The rods are suspended in sucrose solutions of sufficient density to keep them afloat. Measuring the absorption of such suspensions against a sucrose blank yields a spectrum much distorted by light scattering. If, however, he measures the absorption of two suspensions against one another, they are sufficiently stable to yield a level base line. If one now bleaches the one and measures its spectrum against the unbleached sample

‖ Cf. Reference 142.

¶ Cf. Reference 146.

References 149 and 150.

* References 152 and 153.

as a blank, one determines directly the difference spectra of the photosensitive pigment and the product of bleaching. It should be stressed that the spectra so obtained are difference spectra, and can be compared with absorption spectra only if the spectra of the photosensitive substance and its bleached products do not overlap.

Hubbard [154] has measured the respiration of isolated rod outer limbs and finds that they respire actively for many hours, even in the absence of added substrate. Substrates increase the respiratory rate to a level comparable with the respiratory quotient of whole retina. The respiratory rate of dark-adapted outer limbs is not affected by bleaching.

Mechanism of Dark Adaptation and the Visual Threshold.—There has been a burst of activity in this area. For several years investigators have been noticing in a qualitative way that the bleaching of a small amount of rhodopsin can have a large visual effect. Rushton and Cohen [155] have now shown directly that lights of intensities which they calculated could bleach at most 2% of the rhodopsin in the dark-adapted eye, raise the visual threshold ten- to sixtyfold. Furthermore, the rate of dark adaptation, when measured simultaneously in a 3-in. area with a 7-msec. flash of light and in a 2-degree field with a 0.5-sec. flash (both 10 degrees from the fovea), is slower in the small field-short flash situation. The threshold therefore is clearly not a simple function of the concentration of visual pigment. The authors believe that by far the most important factor in dark adaptation is an increase in the quantum sensitivity of the retina. In a subsequent communication, Rushton and Campbell [156] show by direct measurement of the rhodopsin level in the human eye [157] that the rhodopsin concentration is not changed appreciably by the light adaptations used in the above experiment. They therefore conclude that "changes in visual sensitivity cannot be due to the alteration in the chance of a quantum being absorbed; it must be entirely due to a change in the efficacy of the quantum when it is absorbed." They find that when rhodopsin in the human eye is bleached with bright lights, it is resynthesized, with a half-return constant of about seven minutes. At moderate room illuminations, only about 10% of the rhodopsin is bleached at equilibrium.

Wald [158] has confirmed the observation that the bleaching of only a very small amount of rhodopsin is associated with a large rise in threshold and has proposed a new theory connecting these two functions. He assumes that a receptor is a compartmental structure, each compartment responding in an all-or-nothing way to the absorption of a first-light quantum. Further quanta absorbed in an already discharged compartment do not alter the threshold; they merely slow recovery (i. e., dark adaptation), since the compartment has to be completely charged before it can fire again. Wald has measured the visual threshold and the bleaching of rhodopsin solutions under identical conditions of illumination and finds that when 0.006% of the rhodopsin is bleached, the threshold rises 8.5 times; when 0.19% is bleached, the threshold rises 480 times, and when 0.59% is bleached, it rises 3300 times. When these percentages are converted to approximate numbers of rhodopsin molecules bleached per rod, these turn out to be 1,200, 40,000, and 120,000, respectively. The bleaching of a very small percentage of rhodopsin can therefore result in a large rise in threshold if one remembers (1) that the bleaching of one molecule is enough to discharge a completely dark-adapted rod (or a compartment of a rod) and (2) that the rods are tremendously overloaded with rhodopsin. The overloading causes the sensitivity to be very high. The discharge following the absorption of one quantum implies that a negligible change in rhodopsin concentration will have a noticeable visual effect. Wald also discusses in terms of dimensions and rhodopsin content the possibility that the compartments may correspond to the membranes observed by Sjöstrand with the electron microscope † and raises a number

† Cf. Reference 142.

of interesting points bearing on the difference in the functional organization of rod and cone vision.

Denton and Pirenne [159] have measured the visual threshold for large fields (45 degrees) and long exposures (about five seconds), viewed binocularly and without fixation, to give the subject an opportunity to scan the field. In this way, they get very steep frequency-of-seeing curves, implying a variation in sensitivity of no more than 0.2 log unit from one trial to another. At threshold the illumination at 510 mμ corresponds to 1 quantum absorbed every second per about 5000 rods. From the stability of the threshold measurements, they calculate that the spontaneous decomposition of rhodopsin in situ must be about 1000 times as low as that in vitro to yield a "signal-to-noise" ratio consistent with this degree of sensitivity. The stability of such measurements has been explored further by Crawford and Pirenne,[160] who have set up conditions in which the frequency-of-seeing curves at threshold are even steeper, implying a biological variability of about 0.1 log unit. Brindley,[161] furthermore, has calculated that the steepness of such frequency-of-seeing curves implies that at least 4 quanta must be absorbed by 4 different units at the threshold. Any fewer would yield much more variable results.

We have mentioned above that the quantum sensitivity is very high at the threshold but decreases as the eye becomes light-adapted. Wald's compartment theory offers a photochemical explanation for this effect. But there is also some evidence that the organization of the neural connections changes during light and dark adaptation. The most direct and convincing data come from experiments of Barlow, FitzHugh, and Kuffler.[162] Working with unanesthetized, decerebrate cats, they found that the responses of individual ganglion cells in the retina change during dark adaptation. Units whose receptive field in the light-adapted eye consist of an "on" response at the center ("on-center" units) and an "off" response in the periphery change during dark adaptation to "on" units throughout. Similarly, "off-center" units with an "on" peripheral field yield only "off" responses when dark-adapted. While in the light-adapted state the periphery exerts an inhibitory action on the center, this is removed during dark adaptation, and the periphery actually begins to summate with the center.

Willmer [163] has noted a decrease in the summation areas for blue, as opposed to red and yellow receptors, in the fovea as one goes from the threshold to higher brightness levels in human color discrimination experiments. Arden and Weale [164] have measured dark adaptation with very small fields (2.7 minutes) foveally and in the periphery and have compared it with dark adaptation measured in larger fields (15 minutes in the fovea and 7 degrees in the periphery). They find that in the small field the dark-adapted threshold of the peripheral rods is about the same as that for foveal cones, from which they conclude that the intrinsic thresholds of a rod and a foveal cone are about the same. The peripheral cones, however, still appear to have their threshold about 2.3 log units above the rod threshold, implying that their intrinsic sensitivity is about 500 times as low as that of the foveal cones. When the test stimulus covers a larger area, the peripheral rod threshold drops below the foveal cone threshold. The separation of peripheral cone and rod thresholds, however, remains about the same. Arden and Weale believe that the fall in threshold is due primarily to an increase in summation. In that case the efficiency of summation would have to be the same for the two types of receptor. Arden and Weale [165] have also measured the variation of the latent period of vision with brightness in 2.7-minute fields foveally and in the periphery by comparing the latencies in the two eyes, one of which is exposed to a constant intensity while the intensity of the stimulus is varied in the other eye. They find that in the fovea the latency is minimal about 2.5 to 3 log units above the threshold and increases slightly (about 10 msec.) toward higher and somewhat more (about 30 msec.) toward lower brightnesses. In the periphery, as compared with the fovea, the

latency decreases steadily from threshold to about 4 log units above the threshold, over a range of about 100 msec. It is puzzling that the peripheral function is quite smooth, since about 2.3 log units above the threshold there must be a shift from rod to cone vision (viz., 24 above). Extrapolation of the data yields the same latencies in the fovea and periphery about 6 log units above the threshold.

Van den Brink and Bouman [166] have measured spatial summation up to 30 minutes of arc, and temporal summation up to 0.3 second with two stimuli of 0.01-second duration and 1-minute diameter in the fovea and at eccentricities up to 10 degrees. They find that temporal summation decreases with light adaptation foveally and peripherally. Spatial summation in the fovea appears not to depend on adaptation but becomes progressively more dependent as one moves into the periphery, up to 4 degrees, after which the dependency is constant.

In summary, there is general agreement that the visual threshold is not a simple function of the total concentration of photosensitive pigment, but that the quantum demand decreases as the eye becomes dark-adapted; in other words, for a constant visual effect, fewer quanta have to be absorbed at the threshold than in higher states of light adaptation. Wald's compartment hypothesis explains this effect on the basis of the photochemical organization of the individual receptors. But the nervous organization of the retina also changes with the state of adaptation. A complete explanation of the adaptive process will have to take account of both types of phenomena.

Spectral Sensitivity.—By means of dark-adaptation measurements, Auerbach and Wald [167] have been able to demonstrate the presence of a type of cone in the retinal periphery (and the fovea [168]) which is selectively sensitive in the violet region of the spectrum, and the excitation of which stimulates a violet or blue sensation. They adapt the eye to very intense yellow to red lights, which spare this receptor, and then measure dark adaptation with test stimuli of low wave length. Under these conditions, for the first two or three minutes of dark adaptation, the retina is more sensitive to violet light than to yellow. The spectral sensitivity during this interval exhibits a high, narrow peak, maximal at about 436 mμ, in addition to the usual cone vision, maximal at about 555 mμ. After two to three minutes, the retina becomes more sensitive to yellow light than to violet, as is normal in cone vision, and the spectral sensitivity curve acquires the usual shape. The spectral sensitivity of the violet receptor is comparable to Stiles' "blue mechanism." Following blue-light adaptations, the violet receptor is light-adapted, and the spectral sensitivity after one minute follows a smooth curve, with less of a hump in the blue than usual. The hump around 436 mμ in the spectral sensitivity curve of the peripheral cones is therefore due to the violet receptor. In the fovea it is exaggerated by the presence of macular pigment.

Naylor and Stanworth [169] have confirmed Wald's earlier observation that the macular pigmentation is due to xanthophyll and have shown that its molecular orientation in the retina is responsible for the Heidinger phenomenon. Brindley [170] has shown that visual acuity in the fovea is higher for red and green lights than for blue, and that the summation area is much larger for blue lights. He therefore suggests that the "red" and "green" mechanisms of the fovea may have individual neural connections via midget bipolar cells, and that the "blue" mechanisms converge via flat or brush bipolar cells.

Gunter [171] has measured the spectral sensitivity of light-adapted cats by a behavorial technique and finds two maxima of approximately equal sensitivity at about 470 and 555 mμ. Denton and Pirenne [172] have measured the spectral sensitivity of the toad Xenopus laevis by two reactions: melanophore expansion and "escape from light." Both yield similar functions with a hump maximal about 560 mμ. The shape and position of the curve correspond to the absorption spectrum of none of the known visual

pigments and may be a composite function.‡ Armington [173] has measured the spectral sensitivity of the turtle with the ERG and found curves maximal around 630 mμ. He also measured the sensitivity of one dark-adapted turtle by a behavioral method and found it to be maximal about 520 mμ, with a hump about 630 mμ. He therefore thinks that the dark-adapted turtle may be using rods.

Photosensitive Pigments.—Crescitelli and Dartnall [174] have extracted from carp retinas a photosensitive pigment absorbing maximally at 520 mμ, and Dartnall [175] has extracted a pigment from Xenopus with an absorption maximum at 519 mμ.§ Both extracts appear to contain primarily porphyropsin. The authors, however, prefer to distinguish between them on the basis that their difference spectra (i. e., the difference between the absorption before and after bleaching; see discussion above) are at 525 and 533 mμ, respectively. They argue that the difference spectrum is of significance in comparisons with the spectral sensitivity function. This, however, is not the case, since the difference spectrum is determined by differences in the absorption spectra of porphyropsin and of retinene$_2$ and opsin. Retinene$_2$ in the eye is rapidly converted to vitamin A$_2$, which, being colorless, exerts no effect on the absorption properties of porphyropsin. The spectral sensitivity function is therefore governed by the absorption spectrum of the photosensitive pigment, and not by its difference spectrum in the presence of retinene.

In the extracts from Xenopus retinas, Dartnall also finds a small amount of a secondary pigment with an absorption maximum at 570 mμ. He believes that this may be the pigment responsible for the spectral sensitivity of Xenopus, measured by Denton and Pirenne.[172]

Arden [177] describes a narrow-band photosensitive pigment with its maximum at about 535 mμ in suspensions of frog rods. His method of measurement, however, is open to criticism. The spectra are again difference spectra measured in rod suspensions which have lost the capacity to convert their retinene to vitamin A.‖ The spectra of retinene and rhodopsin, particularly in rods, overlap considerably; i. e., the rods before bleaching are red and after bleaching orange. Any change in the absorption spectrum of the liberated retinene therefore exerts a considerable effect on the shape of the difference spectrum. Now Arden's technique consists in bleaching the rods with lights containing predominantly 560, 530, or 480 mμ and with white light. The first two yield a difference spectrum at longer wave lengths than the 480 mμ or white light bleaches. This is precisely what one would predict for a difference spectrum, since rhodopsin, when it is bleached by lights containing only wave lengths above about 500 mμ, liberates all-trans retinene, which is isomerized by blue or white light. The isomerate absorbs light at shorter wave lengths than all-trans retinene; the difference spectrum of rhodopsin will, therefore, be shifted correspondingly. The errors inherent in this method are such that we think it should be avoided whenever possible; we wonder why Arden's experiments were not performed with rods capable of reducing their retinene to vitamin A, in which case his difference spectra would have had the validity of absorption spectra and the above-noted complications could have been completely avoided.

Wald, Brown, and Smith [178] have found two red-sensitive pigments in marine and freshwater fishes respectively. The former absorbs maximally at 520-530 mμ and liberates retinene$_1$ on bleaching. The latter has its absorption maximum at about 550 mμ and releases retinene$_2$. The new pigments contribute only about one-tenth of the total photosensitive material in these extracts. The main pigments are still respectively rhodopsin and porphyropsin.

Histochemistry.—Wislocki and Sidman ¶ have published two classic papers describing

‡ See Reference 175.

§ Cf. Reference 176.

‖ Cf. References 152 and 153.

¶ References 179 and 180.

the histochemistry of the retina, particularly the visual cells of frog, Amphiuma, turtle, chick, cat, and rhesus monkey. As they contain much descriptive material and fine photographs, it is impossible to do them justice in a short review. I shall, therefore, mention just a few points. In agreement with Sjöstrand's observations,[142] the authors find large amounts of mitochondria in the ellipsoids of all species studied. This finding is confirmed by Carasso[181] in a number of anurans. Sidman and Wislocki feel that the inner limbs are homologous to the ordinary cell cytoplasm, as they contain all the standard enzymes. The outer limbs are special in their staining: They are strongly acidophilic and Sudanophilic, stain strongly with the periodic acid-Schiff reagent and with stains for sulfhydryl, and are birefringent and fluorescent. RNA occurs exclusively in the myoids. Gourévitch # has studied the glycogen content of fish retinas histochemically and observes a decrease in the glycogen after prolonged light adaptation, which he considers an indication of an increased metabolic rate in the light. The light, however, has to be on continuously for 15 hours before the effect is noticeable, raising a host of questions about the physiological significance of these observations.

Hara and Hara[184] have measured the incorporation of P^{32} into frog retinas. The distribution is quite different when P^{32} is injected into the frog and measured in the retina 36 hours later than when the excised retina is incubated in a medium containing P^{32}. In the former case, the rods and cones contain little P^{32}; in the isolated retina they take it up in large amounts. Preillumination causes an increase in the uptake of P^{32} by isolated retina. The difference between the in vivo and in vitro uptakes leads one to wonder, however, whether the permeability in vitro is not increased, due to cell damage.

Vitamin A and Carotenoids.—Abdullah, Morcos, and Salah[185] have examined the livers of a number of Nile fishes and found vitamin A_2 in all except Anguilla aegyptica, a migratory salt-water spawner, and Tilapia nilotica, a fish living in the Nile Delta. This finding therefore agrees with Wald's generalization that vitamin A_2 is associated with a fresh-water habitat, while marine fishes or fishes which spawn in the sea have vitamin A_1.

Grangaud and co-workers[186] find a factor in the shells and hepatopancreas of summer shrimps which cures xerophthalmia in vitamin A-depleted rats without promoting growth. They have shown that this is not due to contamination with vitamin A or carotene, but that the activity probably resides in astaxanthin. This should certainly be confirmed with crystalline astaxanthin.

Reviews.—Two thorough and very readable reviews have been published by Wald[187] and by Collins.[188] Wald's article discusses possible mechanisms of excitation, in addition to reviewing the chemistry of the rhodopsin, porphyropsin, and iodopsin systems. Collins' review is concerned mainly with the synthesis and bleaching of rhodopsin.

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News and Comment

GENERAL NEWS

Sterilization of Tonometers: Suggested Use of Sterilized Test Block and Inert Discs.—A recent issue of the ARCHIVES has two excellent articles (Driesler, K. K.: Sterilization of Tonometers, A. M. A. ARCH. OPHTH. **53:** 860 [June] 1955. Rosner, R. S.: New Germicidal Tonometer Stand, A. M. A. ARCH. OPHTH. **53:** 889 [June] 1955) concerning the very important subject of sterilization of tonometers. Driesler's method is evidently 100% effective in sterilizing the hand tonometers, but the author, in a personal communication, states that it cannot be used to sterilize the electronic tonometer. Rosner's method may be the answer to the sterilization of the electronic tonometer.

It would seen that the sterilization of the test block has been neglected.

We use electronic tonometers in our office, and it is necessary to adjust the calibration previous to each application of the tonometer. Recently we purchased two extra glass test blocks from V. Mueller and Company. The test blocks are kept in small metal baskets. The blocks are not touched by hand, but the basket containing the test blocks is lifted in and out of the sterilizer by the basket handle. If the electronic tonometer does not touch the metal basket, the calibration is not effected.

There are many solutions that could be used in the eye to protect the cornea, such as methylcellulose (Methocell), methylcellulose with sodium sulfacetamide, and castor oil, but their use would tend to gum the working parts of the instrument.

We have played with the idea of having 10 by 11 mm. sterilized discs made of cellophane, paper, or some other inert material placed on the cornea before taking the tension in order to protect the cornea from abrasions and infections; in fact, we tried sterilized discs of tissue paper, but, unfortunately, the chemicals in the paper caused defects in the corneal epithelium, as shown by punctate staining of the cornea.

Since beginning this article, we find that Horlick, in the April, 1955, issue of the ARCHIVES, page 614, has recommended the use of latex to protect the cornea.

We believe that the use of the sterilized test block will decrease the incidence of infection; furthermore, we believe the use of sterilized discs should be investigated and put into practice if found feasible.

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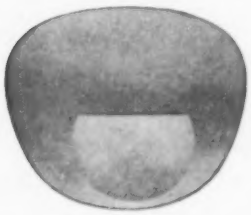

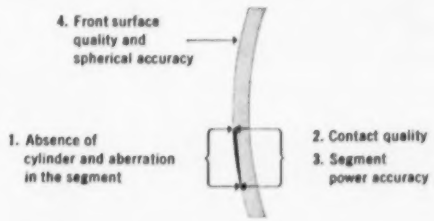
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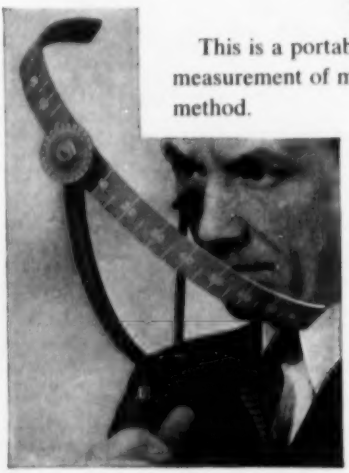

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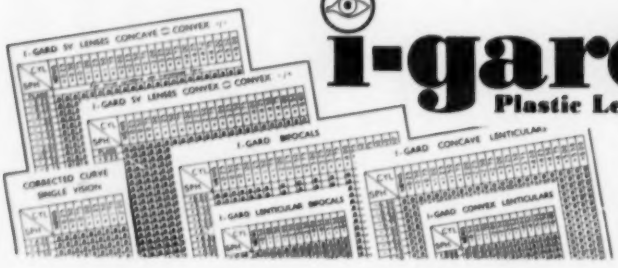

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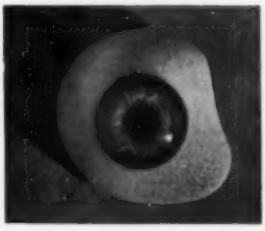

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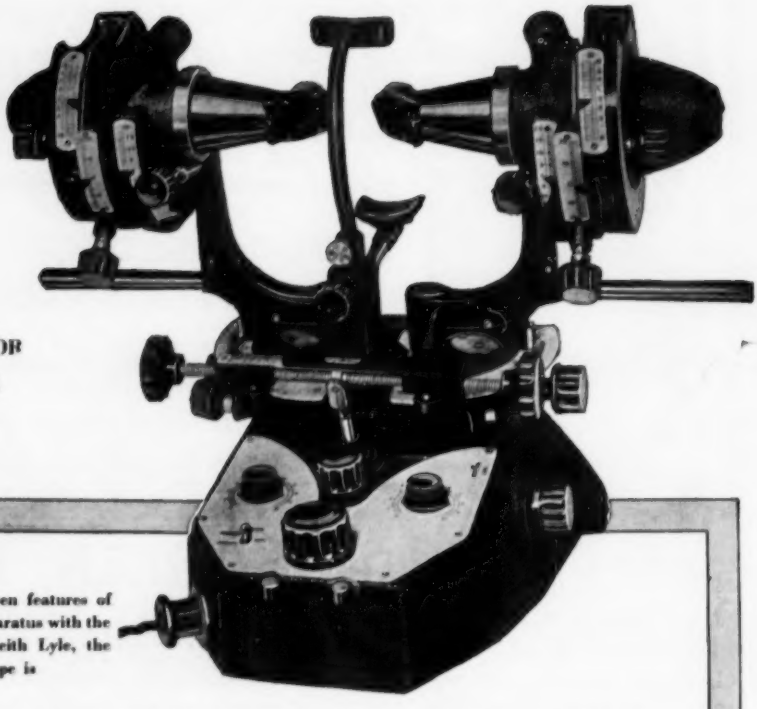

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